PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 November 1998 (19.11.98)	
International application No. PCT/AU98/00292	**Applicant's or agent's file reference** PO6388/JMS
International filing date (day/month/year) 23 April 1998 (23.04.98)	**Priority date** (day/month/year) 23 April 1997 (23.04.97)
Applicant RISBRIDGER, Gail, Petuna et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   22 October 1998 (22.10.98)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Pamella AMALLO-ELOTU

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PO 6388/JMS	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 98/00292	International filing date *(day/month/year)* 23 April 1998	(Earliest) Priority Date *(day/month/year)* 23 April 1997

Applicant
(1) MONASH UNIVERSITY
(2) RISBRIDGER, Gail Petuna et al.

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I)

2. [ ] **Unity of invention is lacking** (See Box II)

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application

   [ ] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed

   [ ] transcribed by this Authority

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

INHIBIN MODULATION OF CELL GROWTH

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure

   [ ] because this figure better characterises the invention

   [X] None of the figures

B x III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention relates generally to a method of modulating cell growth and more particularly, to a method of modulating prostrate cell growth by administering inhibin, an inhibin antagonist or an agent that modulates the expression of inhibin. Even more particularly, the present invention provides a method of treating prostate cancer by inhibiting division of malignant prostate cells. The present invention also relates to a method of screening for a mammal having prostate cancer or a predisposition to prostate cancer.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00292

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 38/22, G01N 33/74, C12Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K, G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: inhibin; activin and prostat:
MEDLINE: inhibin and prostate and growth; (inhibin* or activin) and prostatic neoplasms
CAPLUS: inhibin/RN/THU and prostat?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93/25224 A (VETROGEN CORPORATION) 23 December 1993 entire document	1-67
X	WO 89/11862 A (BIOTECHNOLOGY AUSTRALIA PTY. *et al.*) 14 December 1989 entire document	1-57
X	WO 86/06076 A (BIOTECHNOLOGY AUSTRALIA PTY. LTD. *et al.*) 23 October 1986 entire document	1-57

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
20 May 1998

Date of mailing of the international search report
27 MAY 1998

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200
WODEN ACT 2606
AUSTRALIA
Facsimile No.: (02) 6285 3929

Authorized officer
T. SUMMERS
Telephone No.: (02) 6283 2291

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00292

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 86/00078 A (MONASH UNIVERSITY *et al.*) 3 January 1986 entire document	1-57
X	WO 91/10445 A (GENETECH, INC.) 25 July 1991 entire document	1-57
X	WO 91/10446 A (GENENTECH, INC.) 25 July 1991 entire document	1-57
X	WO 92/04913 A (CHILDREN'S HOSPITAL MEDICAL CENTER OF NORTHERN CALIFORNIA) 2 April 1992 entire document	1-57
X	EP 617966 A1 (PERRINE S.P. *et al.*) 5 October 1994 entire document	1-57
X	Ying S.Y. *et al.*, "Expression and localization of inhibin/activin subunits and activin receptors in the normal rate prostate", *Life Sci.* (1997), 60(6), 397-401 entire document	1-57
X	Ying S.Y. *et al.*, "Activins and activin receptors in cell growth", *Proc. Soc. exp. biol. Med.* (February 1997), 214(2), 114-122 entire document	1-57
X	Wang Q.F. *et al.* "Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP", *Endocrinology* (December 1996) 137(12), 5476-5483 entire document	1-57
X	Dalkin AC *et al.*, "Activin inhibition of prostate cancer cell growth: selective actions on androgen-responsive LNCaP cells", *Endocrinology* (December 1996), 137(12), 5230-5235 entire document	1-57
X	Risbridger GP *et al.*, "Inhibin-related proteins in rat prostate", *J. Endocrinol.*, (April 1996), 149(1), 93-99 entire document	1-57

AUSTRALIAN PATENT OFFICE
SEARCH REPORT

Application No.
AU 98/00292

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Zhang Z *et al.*, "Regulation of growth and prostatic marker expression by activin A in an androgen-sensitive prostate cancer cell line LNCaP", *Biochem. Biophys. Res. Commun.*, (19 May 1997), 234(2), 362-365 entire document	1-57
P,X	McPherson S.J. *et al.*, "Growth inhibitory response to activin A and B by human prostate tumour cell lines, LNCaP and DU145", *J. Endocrinology* (September 1997), 154(3), 535-545 entire document, especially page 542 column 2 last paragraph - page 544 first paragraph	1-67
P,X	Thomas T.Z. *et al.*, "Expression and localization of activin subunits and follistatins in tissues from men with high grade prostate cancer", *J. Clin. Endocrinol. Metab.*, (November 1997), 82(11), 3851-3858 entire document, especially page 3857, column 1 last paragraph	1-67
P,X	Mellor S.L. *et al.*, "Loss of the expression and localization of inhibin alpha-subunit in high grade prostate cancer *J. Clin. Endocrinolol. Metab.* (March 1998), 83(3), 969-975 entire document	1-67
X	Matzuk M.M. *et al.*, "α-inhibin is a tumour-suppressor gene with gonadal specificity in mice", Nature (26 November 1992),360(6402) 313-319 entire document	1-67

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00292

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9325224	AU	43045/93	EP	648126	US	5428011
WO	8911862	AU	37591/89	EP	368992	US	5196192
		WO	8911862				
WO	8600078	AU	44374/85	DK	605/86	EP	185034
		IL	75412	JP	8048636	NO	860427
		NZ	212248	US	5102807	US	5364837
		ZA	8504346				
WO	9110445	AU	71734/91	EP	509040		
WO	9110446	AU	70783/91	EP	509047	US	5102868
WO	9204913	AU	87613/91	EP	548276		
EP	617966	CA	2001897	EP	367590	IL	92167
		JP	2256622	PT	92174	US	4997815

END OF ANNEX

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PO6388/JMS

Box No. I TITLE OF INVENTION

MODULATION OF CELL GROWTH AND METHODS RELATING THERETO

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

MONASH UNIVERSITY
Wellington Road
Clayton 3168
VICTORIA
AUSTRALIA

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

RISBRIDGER, Gail Petuna
14 Coppin Street
East Malvern 3148
VICTORIA
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BRITAIN

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SLATTERY, John M
HUGHES, E John L
CORBETT, Terence G
Davies Collison Cave
1 Little Collins St
Melbourne 3000
VICTORIA
AUSTRALIA

Telephone No. +61 3 9254 2777

Facsimile No. +61 3 9254 2770

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

LſU.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

DE KRETSER, David Morritz
1 Leura Street
Surrey Hills 3127
VICTORIA
AUSTRALIA

This person is:
- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania
- [X] AM Armenia
- [X] AT Austria
- [X] AU Australia
- [X] AZ Azerbaijan
- [X] BA Bosnia and Herzegovina
- [X] BB Barbados
- [X] BG Bulgaria
- [X] BR Brazil
- [X] BY Belarus
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China
- [X] CU Cuba
- [X] CZ Czech Republic
- [X] DE Germany
- [X] DK Denmark
- [X] EE Estonia
- [X] ES Spain
- [X] FI Finland
- [X] GB United Kingdom
- [X] GE Georgia
- [X] GH Ghana
- [X] GM Gambia
- [X] GW Guinea-Bissau
- [X] HU Hungary
- [X] ID Indonesia
- [X] IL Israel
- [X] IS Iceland
- [X] JP Japan
- [X] KE Kenya
- [X] KG Kyrgyzstan
- [X] KP Democratic People's Republic of Korea
- [X] KR Republic of Korea
- [X] KZ Kazakhstan
- [X] LC Saint Lucia
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LS Lesotho
- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova
- [X] MG Madagascar
- [X] MK The former Yugoslav Republic of Macedonia
- [X] MN Mongolia
- [X] MW Malawi
- [X] MX Mexico
- [X] NO Norway
- [X] NZ New Zealand
- [X] PL Poland
- [X] PT Portugal
- [X] RO Romania
- [X] RU Russian Federation
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia
- [X] SK Slovakia
- [X] SL Sierra Leone
- [X] TJ Tajikistan
- [X] TM Turkmenistan
- [X] TR Turkey
- [X] TT Trinidad and Tobago
- [X] UA Ukraine
- [X] UG Uganda
- [X] US United States of America
- [X] UZ Uzbekistan
- [X] VN Viet Nam
- [X] YU Yugoslavia
- [X] ZW Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] CY Cyprus
- [ ]
- [ ]

In addition to the designations made above, the applicant also makes under Rul 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) AUSTRALIA	(23-4-1997) 23 April, 1997	PO6388	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

B x N . VIII CHECK LIST

This international application contains the foll wing number of sheets:

1. request	4	sheets
2. description	58	sheets
3. claims	7	sheets
4. abstract	1	sheets
5. drawings	8	sheets
Total	78	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

MONASH UNIVERSITY
By: Peter L. Darvall

Name: Peter Leo Darvall
Position: Deputy Vice-Chancellor

RISBRIDGER, G P

DE KRETSER, D M

For receiving Office use only

1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal f search copy delayed until search fee is paid

2. Drawings:
☐ received:
☐ not received:

For International Bureau use only

Date of receipt of the record copy

WO 98/47526
PCT/AU98/00292

PATENT COOPERATION TREATY TUESDAY, 10 NOV 1998

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
SLATTERY, John, M.
Davies Collinson Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year) 29 October 1998 (29.10.98)	
Applicant's or agent's file reference PO6388/JMS 2045668	**IMPORTANT NOTICE**
International application No. PCT/AU98/00292	**International filing date** (day/month/year) 23 April 1998 (23.04.98)
Priority date (day/month/year) 23 April 1997 (23.04.97)	
Applicant MONASH UNIVERSITY et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BY,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,GM,GW,HU,ID,IS,KE,
   KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NZ,OA,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,
   TM,TR,TT,UA,UG,UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   29 October 1998 (29.10.98) under No. WO 98/47526

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimil N . (41-22) 740.14.35

Authorized fficer
J. Zahra

Telephone N . (41-22) 338.83.38

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/22, G01N 33/74, C12Q 1/68 — A1

(11) International Publication Number: WO 98/47526

(43) International Publication Date: 29 October 1998 (29.10.98)

(21) International Application Number: PCT/AU98/00292

(22) International Filing Date: 23 April 1998 (23.04.98)

(30) Priority Data:
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(71) Applicant *(for all designated States except US)*: MONASH UNIVERSITY [AU/AU]; Wellington Road, Clayton, VIC 3168 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: RISBRIDGER, Gail, Petuna [GB/AU]; 14 Coppin Street, East Malvern, VIC 3148 (AU). DE KRESTER, David, Morritz [AU/AU]; 1 Leura Street, Surrey Hills, VIC 3127 (AU).

(74) Agents: SLATTERY, John, M. et al.; Davies Collinson Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: INHIBIN MODULATION OF CELL GROWTH

(57) Abstract

The present invention relates generally to a method of modulating cell growth and more particularly, to a method of modulating prostate cell growth by administering inhibin, an inhibin antagonist or an agent that modulates the expression of inhibin. Even more particularly, the present invention provides a method of treating prostate cancer by inhibiting division of malignant prostate cells. The present invention also relates to a method of screening for a mammal having prostate cancer or a predisposition to prostate cancer.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P06388/JMS	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. PCT/AU 98/00292	International filing date *(day/month/year)* 23 April 1998	Priority Date *(day/month/year)* 23 April 1997

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[6] C12Q 1/68; G01N 33/74; A61K 38/22

Applicant

MONASH UNIVERSITY (et al)

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **four** sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
22 October 1998	1 March 1999
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer Arati Sardana ARATI SARDANA Telephone No. (02) 6283 2627

Form PCT/IPEA/409 (Cover sheet) (July 1998) arati sardana

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU 98/00292

I. Basis of the report

1. With regard to the **elements** of the international application:*

- [X] the international application as originally filed.
- [ ] the description, pages , as originally filed,
  pages , filed with the demand,
  pages , filed with the letter of .
- [ ] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages , filed with the letter of .
- [ ] the drawings, pages , as originally filed,
  pages , filed with the demand,
  pages , filed with the letter of .
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**58-67**	**YES**
	Claims	**1-57**	**NO**
Inventive step (IS)	Claims	**60-62, 64, 65 and 66**	**YES**
	Claims	**1-59, 63 and 67**	**NO**
Industrial applicability (IA)	Claims	**1-67**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

1 WO 89/11862 (Biotechnology Australia Pty et al) 14 December 1989

2 WO 86/06076 (Biotechnology Australia Pty et al) 23 October 1986

3 WO 86/00078 (Monash University et al) 3 January 1986

4 WO 91/10445 A (Genentech, Inc) 25 July 1991

5 WO 91/10446 A (Genentech, Inc) 25 July 1991

6 WO 92/04913 A (Children's Hospital medical Centre of Northern California) 2 April 1992

7 EP 617966 A1 (Perrine, S P et al) 5 October 1994

8 Ying, S Y et al, "Expression and Localisation of Inhibin/activin Subunits and Activin Receptors in the Normal Rat Prostate", Life Sci (1997), 60(6), 397-401

9 WO 93/25224 A (Vetrogen Corporation) 23 December 1993

10 Wang, Q F et al, "Activin Inhibits Basal and Androgen-stimulated Proliferation and Induces Apoptosis in the Human prostatic Cancer Cell Line LNCaP", Endocrinology (December 1996) 137(12), 5476-5483

The above documents 1 to 7 and 9 disclose modulation of cell growth in a mammal and method of treating mammals by administering cell growth regulators inhibin and activin, both members of transforming growth factor β family. These documents further disclose administration of inhibin antagonist to mammals and pharmaceutical compositions comprising inhibin, α-inhibin, activin and inhibin antagonist.

Due to the above disclosure claims 1, 7, 12, 14, 20, 25, 27, 33, 38, 40, 46, 51, 53, 55, 57 and claims appended thereto are rendered not novel by the citations 1 to 7 and 9.

Ying et al, discloses absence of inhibin in human prostatic cancer cells.

In light of the above disclosure it would be obvious for a skilled person to screen for down regulation of inhibin protein levels or down regulation of inhibin gene expression to identify prostate cancer or predisposition to prostate cancer in mammals. Therefore claims 58, 59 and 63 lack an inventive step.

Citation 10 Wang et al, discloses that the reduced activin biosynthesis is an indicator of human prostate cancer.

In the light of this disclosure it would be obvious for a skilled person to screen for the modulation of the activin protein

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : Box V

as an indicator of mammalian prostate cancer or a predisposition to prostate cancer. Therefore, claim 67 lacks an inventive step.

Please note that claims 1-26, 58, 59, 64, 66 and 67 are subject matter of Rule 67.1 (method of treatments of humans) and as such do not require an international preliminary examination. However, because the subject matter does not contravene Australian law these claims have been considered in this opinion.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To: Agent :

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

WEI ... OCT 1998

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing (day/month/year) 27 OCT 1998 (27/10/98)

Applicant's or agent's file reference
PO6388/JMS 2045668

IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/AU98/00292	23 APR 1998 (23/4/98)	23 APR 1997 (23/4/97)

Applicant

Monash University (et al.)

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

22 OCT 1998 (22/10/98)

2. That date of receipt is:

☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

☐ the date on which this Authority has, in response to the Invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **Attention:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the elections(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide, Volume II.*

☐ *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE **PO BOX 200, WODEN ACT 2606,** **AUSTRALIA**	(Mrs) Cecilia TRACEY
Facsimile No. 02 6285 3929	Telephone No. (02) 6283 2511

Form PCT/IPEA/402 (July 1998)

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/22, G01N 33/74, C12Q 1/68

A1

(11) International Publication Number: WO 98/47526

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Published
With international search report.

(54) Title: INHIBIN MODULATION OF CELL GROWTH

(57) Abstract

The present invention relates generally to a method of modulating cell growth and more particularly, to a method of modulating prostate cell growth by administering inhibin, an inhibin antagonist or an agent that modulates the expression of inhibin. Even more particularly, the present invention provides a method of treating prostate cancer by inhibiting division of malignant prostate cells. The present invention also relates to a method of screening for a mammal having prostate cancer or a predisposition to prostate cancer.

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INHIBIN MODULATION OF CELL GROWTH

The present invention relates generally to a method of modulating cell growth and more particularly, to a method of modulating prostate cell growth. Even more particularly, the present invention provides a method of treating prostate cancer by inhibiting division of malignant prostate cells.

The present invention also relates to a method of screening for a mammal having prostate cancer or a predisposition to prostate cancer.

The bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Disorders of the prostate gland are of particular concern in ageing men. Figures suggest that approximately one in four males above the age of 55 will suffer from a prostate disease in some form. The incidence in Australia of prostatic cancer is high and similarly prevalent rates occur in most communities. This represents a significant cost to health care systems and decreases the quality of life of men suffering from this disorder.

Inhibins are gonadal derived hormones which have a negative feedback action on the release of pituitary follicle stimulating hormone (FSH). They consist of an α and either a β_A or β_B subunit linked by disulphide bonds (Burger *et al.*, 1996). Inhibin A is formed by the dimerisation of α and β_A subunits; inhibin B from dimerisation of α and β_B subunits. The α-inhibin subunit is synthesised in precursor forms consisting of pre, pro, αN and αC components. The precursor α and β subunits link to form a 105 kD bioactive inhibin,

which forms 31-34 kD inhibin αC-β after postranslational modification (cleavage of the pre, pro and αN regions from the α-subunit and pro from the β subunit) (Robertson *et al.*, 1994). Inhibin B is considered to be the physiologically important form of inhibin which regulates FSH release in men (Illingworth *et al.*, 1996). Dimerisation of two β subunits results in the formation of activin. Three dimeric forms of activins have been published, Activin A ($\beta_A \beta_A$), Activin B ($\beta_B \beta_B$) and Activin AB ($\beta_A \beta_B$). In contrast to the inhibins, the activins stimulate pituitary FSH (Ling *et al.*, 1986).

The inhibin β subunits show approximately 30% homology with the β subunits of TGF β and thus, inhibins are members of the TGS β superfamily of growth and differentiation factors (Massague, 1990). In accordance with this classification, the inhibins have been shown to have a wide range of effects, in addition to the regulation of FSH. In erythroid, immune and endocrine tissues, both proliferative and antiproliferative actions of inhibin has been described (Mather *et al.*, 1990; Hedger *et al.*, 1989; Kaipia *et al.*, 1994). Activin A has also been reported to induce apoptosis (Nishihara *et al.*, 1993). In many instances, the actions of inhibins can be antagonised by activins (Hseuh *et al.*, 1987). The actions of activins are mediated through specific serine/threonine kinase receptors (Matthews *et al.*, 1991). No specific receptors for inhibins have been isolated to date. In addition to receptors for activin, there are binding proteins for activins which include follistatins (Nakamura *et al.*, 1990). Follistatins have no structural homology to inhibins or activins but can bind strongly to activins and, in doing so, suppress or neutralise their bioactivity (Mather *et al.*, 1993). Two mRNA species have been identified for follistatin which arise from alternate splicing, and result in two proteins denoted FS288 and FS315. FS288 has been demonstrated to be membrane associated, while FS315 is a secreted protein (Michel *et al.*, 1990).

Franchimont (1982) showed that seminal plasma is a source of inhibin, as plasma from normal men significantly suppressed serum FSH when administered to castrate rates. More recently, it has been reported that the rat ventral prostate gland itself is a site of synthesis of inhibin and related proteins (Risbridger *et al.*, 1996).

Understanding the cellular localisation and expression of inhibin in prostate tissue from men with and without carcinoma of the prostate is required to determine the role of inhibin in prostate cancer. In work leading up to the present invention, the inventors discovered that in tissues from men with benign prostatic hyperplasia, basal cell hyperplasia or in non-malignant regions of specimens from men with prostate cancer, inhibin α-subunit mRNA and protein expression were observed. In contrast, in malignant regions of tissue from men with advanced stage prostate cancer the localisation and expression of inhibin α-subunit was down regulated in that mRNA and protein were not detectable in poorly differentiated tumour cells.

Accordingly, one aspect of the present invention relates to a method of modulating cell growth in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a genetic sequence encoding inhibin.

Reference hereinafter to "inhibin" should be read as including reference to all forms of inhibin and fragments thereof or derivatives, homologues, analogues, mutants and variants thereof including all subunit polypeptides thereof including by way of example any protein encoded by the α or β subunit gene, the monomeric α-subunit polypeptide, the subunit precursor polypeptides pre, pro αN and αC, the monomeric β subunit polypeptide, the dimeric αβ polypeptide (for example $\alpha\beta_A$, $\alpha\beta_B$, $\alpha\beta_C$, $\alpha\beta_D$, and $\alpha\beta_E$) the dimeric precursor αC-β polypeptide and including, but not limited to, derivatives, homologues, analogues, mutants and variants thereof.

Preferably, said inhibin is the α-subunit polypeptide (α-inhibin) or fragment thereof or derivative, homologue, analogue, mutants and variants thereof. Reference to α-inhibin, hereinafter, is not intended to be limiting and should be read as including reference to all forms of α-inhibin including any protein encoded by the α-subunit gene, all subunit polypeptides thereof including by way of example the monomeric subunit precursor polypeptides pre, pro αN and αC, and including, but not limited to, derivatives,

homologues, analogues, mutants and variants thereof.

More particularly, the present invention relates to a method of modulating cell growth in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a genetic sequence encoding α-inhibin.

The term "mammal" includes humans, primates, livestock animals (eg. horses, cattle, sheep, pigs, donkeys), laboratory test animals (eg. mice, rats, rabbits, guinea pigs), companion animals (eg. dogs, cats) and captive wild animals (eg. kangaroos, deer, foxes). Preferably, the mammal is a human or laboratory test animal. Even more preferably, the mammal is a human.

The term "modulating" means up-regulating or down-regulating. Accordingly, although the preferred method is to increase the expression of a genetic sequence encoding α-inhibin, the reduction of the expression of a genetic sequence encoding α-inhibin expression may also be desired under certain circumstances.

The term "expression" refers to the synthesis of a polypeptide utilising the mechanisms of transcription and translation of a nucleic acid molecule.

Although not intending to limit the present invention to any one mode of action, modulation of the expression of a genetic sequence encoding α-inhibin by the administration of an agent to a mammal can be achieved via one of several techniques including but in no way limited to:

(i) introduction of a nucleic acid molecule encoding α-inhibin or a derivative thereof to modulate the capacity of that cell to synthesize α-inhibin;

(ii) introduction into a cell of a proteinaceous or non-proteinaceous molecule which modulates promoter operation of a gene;

(iii) introduction into a cell of a proteinaceous or non-proteinaceous molecule which modulates transcriptional and/or translational regulation of a gene.

Said gene may be an α-inhibin gene or some other gene which directly or indirectly regulates the expression of an α-inhibin gene.

Preferably, expression of a genetic sequence encoding α-inhibin expression is modulated in prostate cells and even more preferably the prostate cells are malignant.

According to this preferred aspect of the present invention there is provided a method of modulating malignant prostate cell growth in a mammal said method comprising administering to said mammal an effective amount of agent for a time and under conditions suffient to modulate the expression of a genetic sequence encoding α-inhibin.

Although not intending to limit the present invention to any one theory or mode of action the basal epithelial cells of the prostate gland are the predominant site of the expression of the α-inhibin gene. The synthesis and production of the α-inhibin subunit protein in prostatic basal epithelium correlates with data demonstrating that the β-inhibin subunit proteins are localised in these cells. Since both inhibin α and β subunits are expressed in the same cells the tissues have the ability to produce αβ dimeric inhibin protein. The observation that α-inhibin mRNA and protein is observed in epithelial cells in benign prostate tissues and basal cell hyperplasia but not in poorly differentiated malignant prostate epithelial cells is consistent with tumour suppressive activity of α-inhibin in the prostate gland.

Accordingly, in a preferred embodiment said expression of a genetic sequence encoding α-inhibin is up-regulated.

In a particularly preferred embodiment, up-regulation of a genetic sequence encoding α-inhibin inhibits cell growth.

According to this most preferred embodiment, the present invention relates to a method of inhibiting malignant prostate cell growth in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to up-regulate the expression of a genetic sequence encoding α-inhibin.

The modulation of cell growth in a mammal via the modulation of the expression of a genetic sequence encoding inhibin can also be achieved by the administration of inhibin to said mammal.

Accordingly, another aspect of the present invention relates to a method of modulating cell growth in a mammal said method comprising administering to said mammal an effective amount of inhibin.

Preferably, said cells are prostate and even more preferably said prostate cells are malignant.

Most preferably cell growth is inhibited.

Accordingly, in a preferred embodiment the present invention relates to a method of inhibiting malignant prostate cell growth in a mammal said method comprising administering to said mammal an effective amount of inhibin.

Yet more preferably, said inhibin is α-inhibin.

It has been observed that there is a high degree of homology between inhibins from different mammalian species. Thus the inhibin used may be derived from any origin including human, primate, bovine, ovine, porcine or other mammalian or animal species. Preferably, the inhibin is recombinant human inhibin.

The term "inhibin" used herein include fragments, said fragments having the functional activity of inhibin and including but not limited to homologues, analogues, mutants, variants and derivatives thereof. This includes homologues, analogues, mutants, variants and derivatives derived from natural or recombinant sources including fusion proteins. Reference to "inhibin" should also be understood to encompass inhibin agonists.

The homologues, analogues, mutants, variants and derivatives may be derived from insertion, deletion or substitution of amino acids in the inhibin. Amino acid insertional derivatives of inhibin used in the present invention include amino and/or carboxylic terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Additions to amino acid sequences include fusions with other peptides, polypeptides or proteins. Typical substitutions are those made in accordance with Table 1:

TABLE 1

Suitable residues for amino acid substitutions

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Ala
Gly	Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

The inhibin of the present invention may be in monomeric or multimeric form meaning that two or more molecules are associated together. Where the same inhibin molecules are associated together, the complex is a homomultimer. An example of a homomultimer is a homodimer. Where at least one inhibin molecule is associated with at least one non-inhibin molecule, then the complex is a heteromultimer such as a heterodimer.

Inhibin suitable for use in the present invention may be the inhibin glycoprotein which has a molecular weight of 31 kD in its dimeric form and is made up of a 20 kD α-subunit and a 14 kD β-subunit. Preferably the inhibin used in the present invention may be that described in Robertson *et al.*, (1985) or Forage *et al.*, or similar.

The inhibin suitable for use in the method of treatment aspect of the present invention is not related to the "inhibin" described in WO93/25224 which is a non-glycosylated protein occurring in two forms having molecular weight of 10.5 kD and 16 kD.

Although the preferred method is to down-regulate cell growth, in particular malignant prostate cell growth, the up-regulation of cell growth may be desired under certain circumstances.

Accordingly, in another aspect the present invention provides a method of modulating cell growth in a mammal said method comprising administering a mammalian cell growth modulating effective amount of an inhibin antagonist to said cells.

Preferably, said cells are prostate cells.

Accordingly, in this preferred aspect the present invention provides a method of modulating growth of mammalian prostatic cells comprising administering a prostatic cell growth modulating amount of an inhibin antagonist to said cells.

The term "modulating" has the same meaning as given above.

The antagonists may be any compound capable of blocking, inhibiting, or otherwise preventing inhibin from carrying out its normal biological functions in prostate cells or tissue. Antagonists include monoclonal antibodies specific for inhibin, or parts of inhibin, and antisense nucleic acids which prevent transcription or translation of inhibin genes or mRNA in mammalian cells. Antagonists also include analogues of inhibin which bind the

inhibin receptors and thereby prevent inhibin from performing its normal biological functions in the prostate. Antagonists in the form of analogues may include those analogues described above. Antisense sequences based on the nucleotide sequences of inhibin disclosed in US Patent 4,740,587 and Forage *et al* (1986) are also contemplated.

The agent, inhibin, or inhibin antagonist used may also be linked to a targeting means, such as a monoclonal antibody, which provides specific delivery of the agent, inhibin or antagonist to the cells.

In a preferred embodiment of the present invention, the agent, inhibin or inhibin antagonist used in the method is linked to an antibody specific for the prostate to enable specific delivery to this organ.

Administration of the agent, inhibin, or inhibin antagonists, in the form of a pharmaceutical composition, may be performed by any convenient means. The agent, inhibin or inhibin antagonists of the pharmaceutical composition are contemplated to exhibit therapeutic activity when administered in an amount which depends on the particular case. The variation depends, for example, on the human or animal and the inhibin or inhibin antagonist chosen. A broad range of doses may be applicable. Considering a patient, for example, from about 0.1 mg to about 1 mg of inhibin or antagonist may be administered per kilogram of body weight per day. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly or other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. The inhibin or part thereof or antagonist may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intraperitoneal, intramuscular, subcutaneous, intradermal or suppository routes or implanting (e.g. using slow release molecules). With particular reference to use of inhibin, these peptides may be administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g. with zinc, iron or the like (which are considered as

salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a binder such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate.

The tumour suppressing action of inhibins may be mediated through specific receptor complexes similar to those described for TGF-β and activin. The existence of receptors for inhibins have not been identified although there is a body of indirect evidence to suggest that such receptors exist (Woodruff *et al.*, 1992; Krummen *et al.*, 1994).

A further aspect of the present invention relates to the use of the invention in relation to human disease conditions. For example, the present invention is particularly useful, but in no way limited to use in inhibiting growth of malignant prostate cells.

Accordingly, another aspect of the present invention relates to a method of treating a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a genetic sequence encoding inhibin.

Preferably, said inhibin is α-inhibin.

Preferably said cells are prostate cells and even more preferably said prostate cells are malignant.

Most preferably expression of a genetic sequence encoding α-inhibin is up-regulated.

Yet even more preferably cell growth is inhibited.

Accordingly, in a preferred embodiment the present invention relates to a method of treating malignant prostate cells in a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to up-regulate the expression of a genetic sequence encoding α-inhibin.

The treatment of a mammal by the administration of an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a genetic sequence encoding inhibin and thereby regulating cell growth can also be achieved by the administration of inhibin to said mammal.

Accordingly, another aspect of the present invention relates to a method of treating a mammal said method comprising administering to said mammal an effective amount of inhibin.

Preferably, said inhibin is α-inhibin.

Preferably said cells are prostate cells and even more preferably said prostate cells are malignant.

Most preferably cell growth is inhibited.

Accordingly, in a preferred embodiment the present invention relates to a method of treating malignant prostate cells in a mammal said method comprising administering to said mammal an effective amount of α-inhibin.

In yet another aspect the present invention relates to the use of an agent capable of modulating the expression of a genetic sequence encoding inhibin in the manufacture of a medicament for the modulation of cell growth in a mammal.

Preferably said cells are prostate and even more preferably said prostate cells are malignant.

Most preferably cell growth is inhibited.

Yet more preferably said inhibin is α-inhibin.

Most preferably, expression of a genetic sequence encoding α-inhibin is up-regulated.

Yet another aspect of the present invention relates to the use of inhibin in the manufacture of a medicament for the modulation of cell growth in a mammal.

Preferably said cells are prostate and even more preferably said prostate cells are malignant.

Most preferably cell growth is inhibited.

Yet more preferably said inhibin is α-inhibin.

A related aspect of the present invention relates to agents for use in modulating the expression of a genetic sequence encoding inhibin wherein modulating expression of said genetic sequence regulates cell growth.

Preferably said inhibin is α-inhibin.

Preferably said cells are prostate cells and even more preferably said prostate cells are malignant.

Most preferably the expression of a genetic sequence encoding α-inhibin is up-regulated.

Yet even more preferably cell growth is inhibited.

In yet another related aspect the present invention relates to inhibin for use in regulating cell growth.

Preferably, said inhibin is α-inhibin.

Preferably said cells are prostate cells and even more preferably said prostate cells are malignant.

Yet even more preferably cell growth is inhibited.

In a related aspect of the present invention the mammal undergoing treatment may be human or an animal in need of therapeutic or prophylactic treatment of a prostate disorder or a potential prostate disorder.

The tumour suppressive function of α-inhibin protein is predicated on the observation that α-inhibin mRNA and protein is present in the prostate basal epithelial cells of patients with benign prostate disease and in said epithelial cells located in non-malignant regions of prostatic tissue from patients exhibiting prostate cancer but not in the malignant regions of said cancerous prostates.

Accordingly, another aspect of the present invention relates to a method of screening for a mammal having prostate cancer or a predisposition to prostate cancer, said method comprising screening for the down-regulation of the inhibin protein levels and/or gene expression in said mammal, wherein the down-regulation of the inhibin protein levels and/or gene expression is indicative of said mammal being predisposed to prostate cancer or having already developed prostate cancer.

Reference to "down-regulation" should be understood to include reference to the complete absence or total loss of protein and/or gene expression.

"Inhibin" has the same meaning as given above and should therefore be understood to include any protein, or fragment thereof, encoded by the α or β subunit gene whether existing as a monomer, multimer or fusion protein. Examples of a multimer include the αβ heterodimer or a heterodimer comprising any protein encoded by the α-subunit gene in association with any other protein.

Preferably said inhibin is α-inhibin.

"α-inhibin" also has the same meaning as given above. Accordingly, reference to α-inhibin includes, by way of example, reference to any protein, or fragment thereof, encoded by the α-subunit gene, whether existing as a monomer, multimer or fusion protein. Proteins encoded by the α-subunit gene include, for example, pre-pro-αN-αC, pro-αC and the cleavage products αN, αC, pre-pro or isoforms thereof.

The α-inhibin proteins which are detectable in the prostates from patients diagnosed with benign prostate hyperplasia or in the non-malignant regions of prostate may comprise, for example, αN and/or αC regions. The present invention is exemplified, but not limited in any way, by referernce to detection of α-inhibin levels via the detection of the αN or αC regions of the α-inhibin protein. α-inhibin proteins comprising αN and/or αC regions are also referred to as precursor α-subunit proteins. The αN and/or αC regions of precursor α-subunit proteins are found to exist either as part of an existing precursor α-subunit protein or in isolation, for example, following cleavage of said region from a precursor α-subunit protein. Precursor α-subunit proteins exist in many forms including, but not limited to, the forms pre- pro- αN - αC and pro-αC. According to this embodiment of the present invention, detection of α-inhibin proteins, including precursor α-subunit proteins, includes the detection of the αN and/or αC regions both in isolation, and as part of one or more of the various forms of precursor α-subunit protein. The αC and αN regions can be detected, either in isolation or as part of a precursor α-subunit protein, using, for example, the polyclonal

antibodies #αC41 and αN320, respectively.

Accordingly, a preferred embodiment of the present invention relates to a method of screening for a mammal having prostate cancer, said method comprising screening for the down-regulation of αC or isoform thereof in said individual, wherein the down-regulation of the αC or isoform thereof is indicative of prostate cancer.

In another preferred embodiment, the present invention relates to a method of screening for a mammal having prostate cancer said method comprising screening for the down-regulation of αN or isoform thereof in said individual, wherein the down-regulation of αN or isoform thereof is indicative of prostate cancer.

In yet another preferred embodiment, the present invention relates to a method of screening for a mammal having prostate cancer said method comprising screening for the down-regulation of α-subunit gene expression in said individual, wherein the down-regulation of α-subunit gene expression is indicative of prostate cancer.

Without limiting the invention to any one theory or mode of action, in the pre-malignant prostate analysis of αC and/or αN, or isoform thereof, expression reveals disruption of the basement membrane and basal cells when compared to a non-cancerous prostate.

Accordingly, a related embodiment of the present invention relates to a method of screening for a mammal having a predisposition to prostate cancer, said method comprising screening for α-subunit gene expression in said individual, wherein α-subunit gene expression reveals disruption of the basement membrane, said disruption indicating a predisposition to prostate cancer.

Although not intending to limit the present invention to any one mode or theory of action, the absence of α-inhibin protein expression in the malignant prostate results in the inability of β subunit protein monomers to form inhibin αβ dimers. Since activin is formed by the dimerisation of two β subunits, modulation of activin levels in the prostate provides an

additional and/or alternative indicator of malignancy.

Accordingly, another aspect of the present invention relates to a method of screening for a mammal having prostate cancer or a predisposition to prostate cancer, said method comprising screening for the modulation of activin protein levels in said mammal, wherein the modulation of activin protein levels is indicative of said mammal being predisposed to prostate cancer or having already developed prostate cancer.

Screening of inhibin, and/or activin protein levels or gene expression in a mammal can be achieved via one of several techniques including but in no way limited to:

(i) *in situ* hybridisation of prostate tissues with probes detecting inhibin αβ dimers or monomers thereof.

(ii) immunohistochemistry of prostate tissues utilising antibody directed any region of to the α monomeric subunit, the β monomeric subunit and/or the αC or αN or isoform of the α monomeric subunit.

(iii) quantitative measurement of the activin and/or inhibin protein in prostate tissue.

(iv) analysis of α or β subunit mRNA expression.

(v) screening of blood to detect αβ dimers or monomers thereof, the α monomeric subunit, the β monomeric subunit, and/or αC or αN or isoform of the α monomeric subunit.

This method is particularly important for prostate cancer.

Preferably, the mammal is human.

In one particularly preferred method, the target inhibin molecules in the biological sample are exposed to a specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with an antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was

present in the sample.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescene and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

In another aspect the present invention relates to a pharmaceutical composition comprising an agent capable of modulating expression of a genetic sequence encoding inhibin thereby regulating cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

Preferably, said inhibin is α-inhibin.

According to this preferred embodiment the present invention relates to a pharmaceutical composition comprising an agent capable of regulating expression of a genetic sequence encoding α-inhibin expression thereby regulating cell growth.

In a particularly preferred embodiment expression of a genetic sequence encoding α-inhibin is up-regulated.

In yet another most preferred embodiment up-regulation of expression of a genetic sequence encoding α-inhibin inhibits cell growth.

Accordingly the present invention relates to a pharmaceutical composition comprising an agent capable of up-regulating expression of a genetic sequence encoding α-inhibin thereby inhibiting cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

Preferably said cells are prostate cells and even more preferably said prostate cells are malignant.

Another aspect of the present invention relates to a pharmaceutical composition comprising inhibin capable of regulating cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

Preferably said inhibin is α-inhibin.

In a particularly preferred embodiment α-inhibin inhibits cell growth.

Accordingly the present invention relates to a pharmaceutical composition comprising α-inhibin capable of inhibiting cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

Preferably said cells are prostate cells and even more preferably said prostate cells are malignant.

These components are referred to as the active ingredients.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed

in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: a binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention is further described by the following non-limiting figures and/or examples.

In the Figures:

Figure 1 is a photographic representation of analysis of α-subunit mRNA by RT-PCR.

Figure 2a is a graphical representation of radioactive profiles of inhibin tracer incubated ± prostate cytosol for 2 days.

Figure 2b is a graphical representation of radioactive profile of activin tracer incubated ± prostate cytosol.

Figure 3 is a graphical representation of logit plots of B/B_0 recombinant human activin standard (Has3) and rat prostate cytosol.

Figure 4 is a photographic representation of the localisation of inhibin α, β_A and β_B subunit proteins to BPH prostate tissue.

(A-C): Prostate biopsy tissue (A) did not exhibit any inhibin α immunoreactivity in the glandular epithelium or the stroma. In contrast, using the same α-subunit antibody, specific immunoreactivity was localised to the stromal cells of a benign mucinous cystadenoma of the ovary (C). Control prostate tissue incubated with anti-mouse IgG did not detect any positive immunoreactivity (B).

(D-F): Prostate biopsy tissues (D:x20 magnification; E:x40 magnification) stained positively using the β_A subunit antibody, and specific immunoreactivity was localised to the glandular epithelium. Note that there was variable staining within the glandular epithelium itself, as indicated by arrows. Control tissue incubated with normal rabbit serum did not show any positive immunoreactivity (F).

(G-I): Prostate biopsy tissue immunostained with the β_B subunit antibody, showed weak immunoreactivity which was localised to the glandular epithelium (G & H). No specific localisation was recorded in control tissue incubated with the anti-mouse IgG antibody (I).

(Scale bar in A represent 20 microns and is applicable to A, B, C, D, F, G and I. Scale bar in E represent 20 microns and is applicable to E, G and H).

Figure 5 is a photographic representation of RT-PCR and Southern analysis of inhibin α, β_A and β_B subunits, the putative activin β_C subunit, the activin type II receptor (ActRII) and FS288 and FS315 mRNA expression in human BPH biopsy samples. mRNA extracted from two groups of human biopsy samples were analysed by RT-PCR and Southern analysis. Total RNA from adult rat testes (t) and adult rat prostate(p) were used as positive controls, water (w) was used as a negative control. The size of the RT-PCR products was confirmed using pGEM DNA molecular weight markers (Promega Biotec, Madison, USA) (m). The expression of the activin receptor, ActRII (A), inhibin β_A subunit (B), and the putative activin β_C subunit (C) were determined in patients a-e (Lanes a-e). Follistatin (D), inhibin β_B (E) and α (F) subunit mRNA expression was determined in patients f-j (Lanes f-j).

Figure 6 is a photographic representation of the localisation of inhibin αC and αN subunit proteins and αmRNA to benign prostatic hyperplasia tissue. The basal cells in the prostatic epithelium of the benign prostate biopsy tissue stained positively using the cytokeratin market antibody (A). Control prostate tissue incubated with mouse IgG did not detect any specific localisation (B). Specific immunoreactivity for inhibin αC protein was detected in basal cells of the prostate epithelium (C). Control tissue incubated with sheep IgG did not show any positive immunoreactivity (D). Both basal cells and secretory epithelium displayed immunoreactivity for inhibin αN protein (E). No specific localisation was recorded in the control tissue incubated with sheep IgG (F). α-inhibin mRNA was expressed in epithelial basal cells in the benign prostate (G) and in one patient, in both basal and secretory epithelial cells (H - note the section has been counterstained). The localisation was detected with the sense probe (I and J).

Figure 7 is a photographic representation of the localisation of inhibin αC and αN subunit proteins and α mRNA to patients with basal cell hyperplasia. Cytokeratin specific antibody identified areas of basal cell hyperplasia in benign prostate tissue (A). Incubation of the control section with mouse IgG showed no specific immunoreactivity (B). The same regions displayed positive immunoreactivity for both αC and αN inhibin protein (C and E, respectively). Control sections incubated with sheep IgG displayed no positive localisation (D and F, respectively). α-inhibin mRNA was positively expressed in basal cell hyperplasia (G). No specific localisation was detected with the sense probe (H).

Figure 8 is a photographic representation of the localisation of inhibin αC and αN subunit proteins and α-inhibin mRNA to non-malignant and malignant regions of prostate tissue from patients with high grade prostate cancer. Inhibin αC protein was localised to the basal epithelial cells in the non-malignant region (A) of the prostate biopsy. The adjacent tumour cells displayed no positive immunoreactivity (B). Specific localisation of αN protein was observed in the secretory epithelium of the non-malignant region (C); the adjacent tumour tissue displayed no positive staining (D). A control section was incubated with sheep IgG and displayed no specific immunoreactivity (E and F). α-inhibin mRNA was expressed in basal epithelial cells in the non-malignant region (G). The adjacent malignant region showed no positive localisation (H). The control section incubated with the sense probe displayed no staining (I and J).

EXAMPLE 1

DETECTION OF INHIBIN SUBUNIT AND RECEPTOR GENE EXPRESSION BY RT-PCR

This Example indicates that inhibin and activin genes as well as activin receptor genes are expressed in the prostate.

Methods:

RNA Extraction

Total cellular RNA was extracted for adult rat prostates according to Chomczinsky and Sacchi (1987 Anal Biochem 162: 156-158) method, and checked for integrity by visualisation of 18S and 28S ribosomal RNA bands following electrophoresis on a 1% formaldehyde/agarose gel.

Oigonucleotide Primers

The oligonucleotide sequences for the reverse transcription-polymerase chain reaction (RT-PCR) were taken from van den Eiijnden-van Raaij *et al* (1992 Dev biol 154: 356-365) and were as follows. The sequence of the downstream primer for inhibin α was 5' AGC CCA GCT CCT GGA AGG AGA T 3' [SEQ ID NO:1] and the upstream primer was 5' TCA GCC CAG CTG TGG TTC CAC A 3' [SEQ ID NO:2]. For these subunits an intron is absent. Predicted fragment sizes were α-subunit 444bp.

The oligonucleotide primers for the activin receptors type II and IIB were designed from the rat sequence data available on Genebank: the sequence for the downstream primer of ActR-II was 5' GGA ATT CGC ACC AAT GAA CTG 3' [SEQ ID NO:3] and the upstream primer was 5' CGG GAT CCA ACT GCT ATG ACA GG 3' [SEQ ID NO:4]. The internal primer used for the southern detection was as follows 5' TAG GAC AAT GTG GCT TCG GGT GG 3' [SEQ ID NO:5]. These primers span the extracellular and transmembrane regions of the gene and the predicted fragment size is 510 bp. The ActR-IIb primers are as follows, downstream 5' AGC CAG CAC CGC GGT GAG 3' [SEQ ID NO:6] and upstream 5' GTG GCT GTG AAG ATC TCC 3' [SEQ ID NO:7]. The internal primer used for the southern

analysis is as follows 5' TGG CTC ATC ACA GCC TT 3' [SEQ ID NO:8]. These primers span the serine kinase domain and the predicted product size is 366 bp.

Reverse transcription

Reverse transcription was carried out using 0.5 µg total RNA mixed with 4U of AMV reverse transcriptase (Promega Biotec, Madison, WI), 20U of RNasin (Promega Biotec, Madison, WI) 1mM dNTP, 1mM $MgCl_2$, 25pmol of the appropriate downstream primer in PCR buffer (Biotec International, Ltd, WA, Australia) to a final volume of 20µl. The solution was incubated at 42°C for 2 hours, then heated to 95°C for 5 min, and cooled rapidly on ice.

Polymerase Chain Reaction

The PCR was performed in an automatic DNA thermal cycler (Corbett Research Mort Lake Australia) as previously described by Saiki *et al.*, (1988 Science 239: 487-491). Briefly 5µl of the RT mixture was added to 0.2mM dNTP, 25pmol of the appropriate upstream primer and 2U of the thermostable *Tth* DNA polymerase (Biotec International Ltd, WA, Australia) in the PCR buffer (Biotec International Ltd, WA, Australia) in a final volume of 20µl. Denaturation was at 95°C for 30 sec, annealing at 56°C for α-subunit, 55°C Act RII and 46°C for ActRIIB for 30 sec, and extension at 72°C for 1 min for a 40 cycle program. The products were then analysed by agarose gel electrophoresis in IX TAE.

Results

The results show that the inhibin α-subunit gene was detected in prostate tissues from rat tissues from day 10-170 days old by RT-PCR (Figure 1). The activin receptor types II was also detected in these tissues. These data suggest that inhibin and activins and activin receptor genes are expressed in the prostate.

EXAMPLE 2
DETECTION OF ACTIVIN/INHIBIN PROTEINS BY RADIOIMMUNOASSAY (RIA)

This Example indicates that activin and inhibin are present in the prostate, and that activin is produced by the prostate itself.

Methods:

Reagents

Phenylmethyl sulfonyl fluoride (PMSF), BSA, and Triton were purchased from Sigma (St Louis MO). Deoxycholate, Tween 20, Sodium chloride and EDTA were purchased from BDH/Merck (Australia). SDS was from Biorad.

Animals

Adult male Sprague-Dawley rats were obtained from the Central Animal House at Monash University and injected with 75mg EDS (Ethane dimethane sulphonate)/kg body weight in a mixture of DMSO and water as previously described (Risbridger *et al* 1987). Animals were killed in a CO_2 charged chamber at between 3 and 59 days after the administration of EDS as specified in the experiments. Castration was preformed under ether anathesia through a midline abdominal incision. The testes were located and the gubemaculum cut to release the epididymis; the testicular artery was ligated and the tested and epididymis removed. The animals were killed in a CO_2 charged chamber 3 days after castration.

Preparation of cytosols

Prostate glands were immediately excised, placed on ice and weighed. A volume of PBS containing 1mM PMSF was added to the tissue in a ratio of between 1:2-40. The samples were aliquoted and frozen prior to radioimmunoassay.

Detection af activin degradation

In order to determine if residual protease activity in the prostate cytosols was able to degrade activin tracer, aliquots of tracer were incubated at 4°C for 48h in the presence of prostate

cytosol or buffer. The incubates were diluted in nonreducing buffer, boiled for 1 min, microfuged for 30s and applied to 15% SDS-acrylamide gels. The resulting gel was sliced into 1mm fractions and the radioactivity measured; the radioactive profiles for buffer controls were compared to those with prostate cytosol and shown to be identical.

Activin radioimmunoassay

The method is based on that previously published by Robertson *et al* (1992 Endocrinology 130: 1680-1687) with minor modification.

Tracer: human recombinant activin A pool G was iodinated by chloramine T and purified by gel filtration and dye affinity chromatography and used in 0.5% BSA + 0.1% Triton in PBS.

Standard: a human recombinant activin A standard (HAS3) was prepared at concentration of 88 ng/ml and serially diluted in PBS + 0.5% BSA.

Antiserum: an ovine antiserum which has been previously used for radioimmunoassay was raised to a recombinant B_A subunit fusion protein and human recombinant activin A and used at a final dilution of 1:120 000 in CTS reagent (0.125M deoxychloate, 5% Tween 20 and 4% SDS). Limited cross reaction of the antiserum with bovine 31 KDa inhibin and human recombinant 34KDa inhibin, TGFB1, MIS and follistatin < 3.3% has been previously reported (Robertson *et al* 1992).

Conditions of assay

A method was devised using delayed tracer addition conditions over 4 days at 4°C. A donkey anti-sheep serum (PBS + 0.5% BSA and 0.01M EDTA) was used to separate bound from free activin tracer.

Inhibin radioimmunoassay

Samples were assayed for inhibin using antiserum 1989 and the procedure published in Robertson *et al* (1988 Mol Cell Endo 58: 1-8)

Results

Activin/inhibin radioimmunoassay

The radioactive profile obtained following incubation of tracer with buffer or prostate cytosol was identical as shown in Figure 2a,b: non specific degradation of the tracer did not occur under these conditions.

The prostate cytosol contained detectable levels of immunoactive activin, which diluted in linear and parallel to that of an activin standard - human recombinant standard 3 (Has3). (Figure 3). Inhibin immunoactivity was also detected in these examples.

EDS studies

The effect of EDS treatment on prostate weight is shown in Table 2 and confirms previous observations from the inventors' laboratory. Note that a significant decrease in prostate weight was observed in these studies, but that prostate weight had returned to control levels by day 30.

The levels of activin in prostate cytosols obtained after EDS treatment are shown in Table 2. The data are recorded as ng/organ and ng/g prostate tissue. There is a significant decrease in activin in the prostate and per unit mass of tissue, indicating that activin levels are responsive to androgen withdrawal. The implication of the changes in concentration of activin may have yet to be determined.

The levels of inhibin are not significantly changed within 3 days of EDS when expressed as levels per unit mass tissue, but are increased thereafter; these data suggest that inhibin is responsive to androgen withdrawal. Alternatively, as the inhibin RIA detects the pro α_C fragment of inhibin α-subunit, the RIA may be measuring an alteration in immunoactive inhibin forms which are responsive to androgens. (Table 2)

Castration Studies

The effect of castration on prostate weight has been previously documented but it is noted here that there is a significant drop in prostate weight 3 days after castration. Activin levels

do not fall significantly within 3 days after castration, and the concentration in the tissue is elevated (Table 3). Changes to inhibin levels are also shown in Table 3.

Measurement in human seminal plasma samples.

Human seminal plasma samples were obtained from semen donors attending the Andrology Clinic Monash Medical Clinic. Samples were diluted and RIA activin levels measured as described, seminal fluid samples did not degrade activin tracer in this assay. Patient samples were also obtained from men undergoing reversal of vasectomy, pre and post-operatively. The results show that the levels of activin in all three groups are not significantly different. These data are consistent with the hypothesis that activin is produced in the prostate and seminal vesicles and the levels measured in seminal fluid are not testicular in origin.

EXAMPLE 3

TISSUE COLLECTION

Tissues for immunolocalisation were obtained from archival needle biopsy material from 14 men who received no androgen therapy. At least five sections from each biopsy were used for immunochemistry, with antibodies of defined specificity as described below. Benign prostatic hyperplasia was confirmed by histologic examination conducted at Melbourne Pathology (Melbourne, Australia).

Ten patients, age range 49-88, who had received no form of androgen therapy, underwent trans-urethral resection of the prostate (TURP) for symptoms of outflow obstruction. Following informed consent, and in accordance with procedures and processes required by the Standing Committee for Ethics at Monash University, prostate needle biopsy tissues were collected at surgery under sterile conditions. The specimens were wrapped in sterile foil and snap frozen in liquid nitrogen, before storage at -70°C. Pathological examination of tissues taken at surgery, confirmed benign prostatic hyperplasia.

Prostate tissues were obtained from a total of 28 patients, which were grouped according to diagnosis into three groups with BPH, basal cell hyperplasia or prostate cancer. Needle biopsies were obtained from 16 patients with BPH, 2 patients with basal cell hyperplasia and 12 patients with prostate cancer (each having a Gleason score grading between seven and ten). None of the patients had received any form of androgen therapy. Two patients with basal cell hyperplasia were identified by histological examinations and diagnosis. The tissues were fixed in 10% buffered formalin and processed in paraffin.

Three micron sections were cut for immunohistochemistry or *in situ* hybridisation as described below.

EXAMPLE 4
ANTIBODIES

An antibody to human α-inhibin was purchased from Serotec (UK) and has previously been used and shown to be specific for the localisation of inhibin α-subunit immunoreactivity (Vliegen *et al.*, 1993).

Antibody (AS #64) was raised against a human β_A subunit fusion protein and human recombinant activin A in sheep. This antibody has been used for the radioimmunoassay of activin A and has no cross reactivity with Mullerian Inhibiting Substance, TGF β and <3.3% cross reactivity with human recombinant inhibin A (Robertson *et al.*, 1992). The radioimmunoassay using AS #64 has been previously used to purify dimeric activin A to homogeneity from ovine amniotic fluid (de Kretser *et al.*, 1994) and for the detection of activin A in biological fluids and samples (McFarlane *et al.*, 1996). This antibody detects both the monomeric and dimeric forms of activin A, and the cross reactivity of the AS #64 with monomeric β_A in the radioimmunoassay was estimated to be 17% (Robertson *et al.*, 1992). To test the specificity of the antibody staining, non-immune serum was used as a control, or the antiserum was preabsorbed with human recombinant activin α Preabsorption of the antisera was achieved by incubating 5μl of undiluted antisera with 1

μg antigen, either human recombinant activin A or inhibin A, in PBS (200μl) at 4°C overnight. The mixture was diluted to a total volume of 1 ml with PBS and centrifuged at 12,000 rpm, and the supernatant decanted and used accordingly.

A polyclonal rabbit antibody to inhibin β_A was obtained from Dr W. Vale, of the Salk Institute. A mouse monoclonal antibody to β_B subunit was kindly provided by Dr J. Mather, Genentech (San Francisco, USA). Both have been previously used in the detection of β subunit proteins in ovarian tumour tissue (Gurusinghe *et al.*, 1995).

The follistatin antisera, AS #202, was raised in an intact adult male New Zealand rabbit to purified bovine 39 kDa follistatin, and showed <0.5% cross reactivity to bovine inhibin A and bovine activin A (Klein *et al.*, 1991).

An antibody to smooth muscle actin was purchased from Dako Corporation.

The polyclonal antibody #α41 was produced by immunisation against recombinant bovine αC inhibin subunit fusion protein, the sheep was boosted with human αC inhibin subunit fusion protein and human recombinant inhibin α This antibody was used for the detection of the αC inhibin subunit and has been used previously to measure α-inhibin levels in serum from normal and postmenopausal women using immunofluorometric assay. A polyclonal antibody #α320 was directed to a fragment (amino acid 1-26) of the fusion protein bovine α N subunit and used to detect the αN subunit. Immunostaining for cytokeratin was performed using the monoclonal antibody NCL-LP34 obtained from Novacastra Laboratories (Newcastle Upon Tyne, UK).

Additional antibodies used for the detection of the α-subunit protein immunoreactivity included α Groome (Serotec) and α Salk (kindly provided by Professor Vale and Dr J Vaughan).

EXAMPLE 5

IMMUNOSTAINING

After dewaxing, human prostate sections were rehydrated and placed in antigen retrieval solution (Dako, CA, USA) for 20 minutes in a water bath at 85°C. The slides were then washed in PBS and preincubated in CAS block for 30 minutes. Activin A was immunolocalised using AS #64 at a dilution of 1:200, or β_A monoclonal (1:100) and incubated overnight at room temperature. Activin B was localised using the β_B monoclonal antibody (1:100), as was follistatin , using the AS #202 (1:100). Controls were incubated with antiserum preabsorbed with human recombinant activin A (1μg/ml), or a mixture of bovine follistatins (35-, 39-, and 45 kDa) purified from follicular fluid (1μg/ml) or with normal rabbit serum. After overnight incubation, the sections were washed in PBS and incubated with biotinylated rabbit antisheep IgG (activin A), sheep antirabbit (β_A subunit), rabbit antimouse IgG (monoclonal β_B) or biotinylated goat antirabbit sera (follistatin) (Vector Laboratories, California, USA, 1:200) for one hour. Actin staining was localised using the actin antibody (1:50) for 1 hour. Sections were washed 3 times with PBS (0.01M phosphate buffered phosphate; pH 7.4) and then incubated with rabbit anti-mouse IgG (1:200) for 1 hour. After 2 washes in Tris buffer (0.1M Tris-HCl: pH 8), the sections were incubated in Extravidin Alkaline Phosphatase (Sigma, St Louis, MO, USA) (1:100) for one hour. The New Fuchsin Substrate Kit (Biogenex, CA, USA) was used for the demonstration of Alkaline phosphatase. After colour development, the sections were washed in distilled water, counterstained in haematoxylin, dehydrated, cleansed in xylene, mounted in DPX and analysed by light microscopy.

EXAMPLE 6
IMMUNOHISTOCHEMISTRY

Sections were dewaxed, rehydrated and placed in Target Retrieval solution (Dako, Carpinteria, CA); antigenic sites were exposed by heating at 70°C for 7 minutes. After washing in 0.01M phosphate buffered saline (PBS; 10 mM PO_4, 154 mM NaCl, pH 7.4), endogenous peroxidase was blocked by 3% H_xO_2 for 30 minutes. Sections were incubated with 0.2% Triton X-100 (Sigma Chemical Co., St. Louis, MO) for 10 minutes and then blocked with 1:1 mixture of CAS block (Zymed, San Francisco, CA) and 10% normal rabbit serum at room temperature for 20 minutes.

Inhibin was localised using the αC polyclonal antibody (1.6μg/ml) and the αN polyclonal antibody 1.9μg/ml). Basal cells were localised using cytokeratin monoclonal antibody (1:100). All antibodies were incubated at 4°C overnight. Controls were incubated with sheep (inhibin) or mouse (cytokeratin) IgG at matched dilution or protein or protein concentration. After overnight incubation the sections were washed in PBS and incubated with biotinylated rabbit anti-sheep IgG (Vector Laboratories, Burlingame, CA; inhibin) or biotinylated rabbit-antimouse IgG (Dako; cytokeratin) for 60 minutes. The secondary antibody was removed and Vectastain Elite ABC Kit (Vector Laboratories) added for 60 minutes. Following further washes with PBS, peroxidase activity was detected using Liquid 3,3' diaminobenzidine tetrahydrochloride (DAB) Substrate Kit (Zymed). The reaction with Mayers' Haemotoxylin (Sigma Diagnostics, St Louis, MO) and Scotts water, dehydrated and coverslipped with DPX (BDH, Poole, England).

EXAMPLE 7
RT-PCR

Poly A+ RNA Extraction from Human Prostate Needle Biopsy Tissue

Poly A+ RNA was extracted directly from the tissues using the Dynabeads™ protocol (Dynal, Oslo, Norway). The poly A+ RNA was eluted in 50μl of sterile DEPC-treated

water and stored at -20°C until used.

Oligonucleotide Primer Design

Oligonucleotide primers for the α, β_A and β_B subunits were designed from human cDNA sequence data obtained from Genbank (acces. #M32755 [27], #X57578 [28] and #M13437 [29]). The oligonucleotide primers were designed to span the single intron, and yield products of 169bp, 336bp and 500bp respectively. β_C primers (Schmitt *et al.*, 1996) are believed to span an intron, based on the homology between the β_A and β_B subunit members, and yield a product of 290bp.

The PCR primers used for detecting ActRII were designed from the mouse sequence data (Mathews *et al.*, 1991) and span the extracellular and transmembrane domains, to yield a product of 510bp. Follistatin primers (Meinhardt *et al.)* were designed to span exons 5 and 6 of the human follistatin sequence and yield two products of 207bp and 470bp corresponding to FS 315 and FS 288, respectively. The deduced precursor sequences bear no homology with the α, β_A and β_B chains. PCR conditions and primer sequences are outlined in Table 4.

Reverse Transcription (RT)

Reverse transcription for all mRNAs was carried out using 20μl of A+ RNA, denatured at 65°C for 5 min, and mixed with 30 U reverse transcriptase (Promega Biotec, Madison, WI, USA), 40 U RNAsin (Promega Biotec, Madison, WI, USA), 15 pmol Oligo $(dT)_{15}$ primer (Promega Biotec, Madison, WI, USA), 1 mM of each dATP, dTTP, dCTP, dGTP (Promega Biotec, Madison, WI, USA) to a final volume of 50μl. The solution was incubated at 42°C for 2 hours, heated to 95°C for 2 min and cooled rapidly on ice.

Polymerase Chain Reaction (PCR)

The PCR was performed in a Perkin Elmer Cetus DNA Thermal Cycler as previously described by Saiki *et al.*, (Saiki *et al.*, 1985). Briefly, 10 μl of the RT mixture was added to 30 pmol of each primer, and 1 U of Ampli Taq DNA polymerase (Roche Molecular

Systems Inc., Branchburg, New Jersey), in 1 x PCR buffer (Roche Molecular Systems Inc., Branchburg, New Jersey) to a final volume of 50 μl. PCR products were analysed by Nusieve GTG agarose gel electrophoresis in 1 x TAE (0.04 M Tris-acetate, 0.001 M EDTA).

Samples from human prostate tissues were Southern blotted and sequence identity confirmed.

EXAMPLE 8

SOUTHERN BLOTTING RT-PCR PRODUCTS

Probe Labelling

Probes were labelled either with DIG or with ^{32}[P] for Southern analyses. Probes for ActRII, β_A and β_C were derived from sequenced PCR products, and were labelled with DIG. Briefly, 25 ng of denatured probe was mixed with 1 x hexanucleotide mix (Boehringer Mannheim GmbH Biochemica, Germany), 1 x dNTP labelling mix (Boehringer Mannheim GmbH Biochemica, Germany), 2 μl 0.25 mM DIG-dUTP (pH 6.5), and 5U of Klenow enzyme (Promega Biotec, Madison, WI, USA) to a final volume of 20μl. The mixture was incubated at 37°C for 1 hour. 20mM of EDTA was added to stop the reaction, before storing the probe at -20°C.

The FS (Michel *et al.*, 1990) α Najmabadi *et al.*, 1993) β_A (Esch *et al.*, 1987) and β_B (Stewart *et al.*, 1986) probes were labelled with ^{32}[P]. Briefly 25 ng of denatured probe was mixed with 1 x hexanucleotide mix (Boehringer Mannheim GmbH Biochemica, Germany), 1 mM of each dATP, 1 mM dGTP, 1 mM dTTP (Promega Biotec, Madison, WI, USA), 4 mCi of ^{32}[P] dCTP (DuPont, NEN Research Products, Boston, MA, USA) and 10 U of Klenow enzyme (Promega Biotec, Madison, WI, USA) to a final volume of 20 μl, and incubated overnight at room temperature. The probe was precipitated after the addition of 3 μg herring sperm DNA (Promega Biotec, Madison, WI, USA), 0.0 M sodium perchlorate, 0.4 vol isopropanol in a final volume of 165 μl and centrifuged at

13,000g for 5 min. Labelling efficiency was determined by scintillation counting (1900 TR Liquid Scintillation Analyser, Packard Instrument Co., Ulgersmaweg, The Netherlands).

DIG Southern Blot Analysis of RT-PCR Products

Southern blot analysis of RT-PCR products was carried out as follows. Membranes were pre-hybridised for 1 hour in prehybridisation buffer (DIG {5x SSC, 0.1% N-laurolysarcosine, 0.02% sodium dodecyl sulfate, 1% Blocking Reagent (Boehringer Mannheim GmbH Biochemica, Germany)} and ^{32}[P] {Rapid Hyb (Amersham Life Science, Buckinghamshire, UK)}), before addition of denatured probe. Hybridisation was carried out for 2 hrs at 65°C, before washing for 15 min, twice with 2 x SSC + 0.1% SDS, and twice 0.5 x SSC + 0.1% DIG labelled probes were detected using anti-DIG antibody conjugated to alkaline phosphatase, followed colorimetric analysis as per manufacturers directions. ^{32}[P] labelled probes were detected by autoradiography using XOMAT AR film (Eastman Kodak Co., NY, USA) with intensifying screens at -75°C.

EXAMPLE 9
IN SITU HYBRIDISATION

Probe Synthesis

Digoxigenin (Dig) labelled riboprobes were prepared using the method outlined in the Boehringer Mannheim riboprobe labelling kit. Rat and human inhibin α-subunit share an 82% homology and riboprobes to both rat and human sequences were used in this study.

Dig antisense and sense cRNA probes (gift from Dr. Moira O'Bryan, Institute of Reproduction and Development, Monash University, Melbourne, Australia) were synthesised from a ~400bp partial rat α-inhibin subunit cDNA cloned into pGem 4Z (Esch *et al.*, 1987). Antisense probes were transcribed from *Eco*RI linearised plasmids with T7 RNA polymerase and sense cRNA was generated from *Hind*III linearised plasmids with SP6 RNA polymerase. The amount of Dig-labelled RNA was determined

by comparison to a Dig-labelled RNA control using dot blot analysis.

An ≈ 400bp PsI/PvuII fragment of the human inhibin α-ubunit cDNA (gift from Biotech, Roseville, NSW, Australia) was subcloned into pGEM 4z. The cDNA corresponds to positions 702-1115 of the published human inhibin α-subunit nucleic acid sequence (Mason *et al.*, 1986). Antisense probes were synthesised by linearising the plasmids with *Hind*III and transcribed with SP6 RNA polymerase. Sense probes were obtained after linearising with *Eco*RI and transcribed with T7.

After dewaxing, sections were washed in 1xPBS (2x5min) and treated with proteinase K (20μg/ml) for 30 min at 37°C. Following digestion sections were washed in PBS containing 0.2% glycine for 5 min followed by 5 min fixation in 4% Paraformaldeyde. Sections were then washed in PBS 2x5 min, equilibrated for 2 min in 0.1M triethanolamine and acetylated in 0.25%acetic anhydride in Triethanolamine for 5 min. After rinsing in PBS prehybridisation was conducted at 42°C for 60 min in hybridisation buffer which contained 50% formamide, 10% dextran sulphate, 1x Denhardts, 5x SSC (sodium citrate, 1x = 0.15M NaCl, 0.015 M Na citrate), 45mM phosphate buffer, hsDNA (200μg/ml; Progema, WI, U.S.A.) and tRNA (500μg/ml, Sigman, Mo, U.S.A.). Riboprobe was diluted in hybridisation buffer to a concentration of 200-1000μg/ml and denatured at 65°C for 10 min to remove secondary structures. Slides were then incubated at 80°C for 10 min and hybridisation was performed under coverslips in a humidified box at 42°C overnight.

Following hybridisation coverslips were removed in 4xSSC and slides were then washed 2x5 min in 2xSSC. An Rnase A digestion (20ug/ml) was performed at 37°C for 30 min followed by SSC washes of increasing stringency, 2x5 min in 1xSSC, 1x20 min in 0.5xSSC at 42°C. The tissues were briefly rinsed in 0.1M Maleic acid/0.15MNaCl (ph 7.5) and non-specific binding was removed with a Blocking Buffer containing 1% Skim milk powder in 0.1M Maleic acid/0.15MNaCl (ph 7.5) for 30 min at RT. Slides were then incubated in Casblock (Zymed) for 20 min at RT. An anti-digoxigenin alkaline

phosphate conjugate antibody (Boehringer) was diluted 1:1000 in Blocking Buffer and sections were incubated overnight at 4°C. After washing 3x10 min in 0.1M Maleic acid/0.15M NaCl, immunoreactivity was detected with NBT/BCIP substrate (NBT/BCIP) one step; Pierce, Rockford, II, USA). After appropriate colour development (1-20 hours) the reaction was halted by immersion in water.

EXAMPLE 10
IMMUNOLOCALISATION STUDIES

The pattern of localisation of inhibin α, β_A, and β_B subunits is shown in Figure 4, and was determined using specific monoclonal and polyclonal antibodies to the α and β subunit proteins. As shown in Figure 4 A and B, no α immunoreactivity could be detected in BPH tissues, although α immunoreactivity was readily detectable in positive control sections of human ovarian benign cystadenoma (Fig 4C). β_A subunit reactivity was predominantly localised to the epithelial tissues (Fig 4D) and it was noted that the staining intensity was variable within, and between, the glandular structures in the same sections (Fig 4D, E). No immunoreactivity was present in the control sections (Fig 4F). Weak, but detectable, immunoreactivity for the β_B subunit was localised to the epithelium in BPH tissues (Fig 4G). There was variability in the intensity of staining for β_B subunit as shown in (Fig 4H, I). Collectively these data demonstrate that the β_A and β_B but not α, subunit proteins can be detected by immunolocalisation in BPH tissues, using these antibodies.

EXAMPLE 11
INHIBIN/ACTIVIN α, β SUBUNIT, FOLLISTATIN AND ActRII mRNA EXPRESSION

As limited amounts of tissue were obtained, two groups of patient tissues were used for analysis of mRNA expression. Patient samples a-e, were used to determine the presence of the activin receptor, ActRII, inhibin β_A and the putative activin β_C mRNA; patent samples f-j were used to determine follistatin, and inhibin α and β_B subunit mRNA expression.

Total RNA from rat testes (Figure 5, Lane t) and rat prostate (Figure 5, Lane p) were used as positive controls for each of the primer pairs.

mRNA expression of ActRII, β_A and β_C in patient samples a-e

The data in Figure 5A-C demonstrate the mRNA for the activin receptor (ActRII), inhibin β_A subunit, and the putative β_C subunit are expressed in human prostate tissue samples a-e. Figure 5A shows the detection of ActRII mRNA in all five biopsy samples from patients a-e (Lanes a-e respectively), and demonstrated the integrity of the extracted mRNA. Inhibin β_A subunit mRNA expression was detected by Southern analysis in three of the five patient samples (Figure 5B, lanes c, d, e), suggesting variability in β_A mRNA expression. Whereas the putative β_C subunit mRNA was detected in all of the patent samples (Figure 5C, Lanes a-e).

mRNA expression of follistatin and inhibin α and β_B subunit in patient samples f-j

mRNA for the activin binding protein, FS 288 was detected in only two of the five biopsy samples (Figure 5D, Lane i and j respectively); however the alternate splice variant, FS 315, was readily detected in all five patent samples (Figure 5D, Lanes f-j respectively). These results confirm the integrity of the mRNA. However, inhibin β_B mRNA expression was weakly detected in two of the five biopsies (Figure 5E, Lanes f and g), thus the ability to detect inhibin β_B subunit mRNA in the human prostate was variable between the patent samples. Inhibin α-subunit mRNA expression was also detected in four biopsy samples f, g, i and j (Figure 5F Lanes f, g, i & j respectively)

EXAMPLE 12

BPH TISSUES

In the glandular epithelial tissue from patients with BPH, basal cells were localised using a specific cytokeratin monoclonal antibody as shown in Figure 6A. No immunoreactivity was observed in the control sections (Figure 6B). Positive immunoreactivity was localised to epithelial cells in tissue sections from 11 patients with BPH using specific antibodies to

αC and/or αN inhibin subunit proteins. As shown in Figure 6C, inhibin αC subunit was readily detected in basal cells (6 of 8 patient tissues) and there was variable immunoreactivity in the luminal secretory cells (2 of 8 patient tissues). No immunoreactivity was present in the control section (6 of 6 patient tissues; Figure 6E). Positive immunoreactivity for the inhibin αN subunit was localised to both the secretory epithelium and basal cells (Figure 6D). No immunoreactivity was detected in the control section (Figure 6F). No immunoreactivity was localised to any patient tissue using the Groome or Salk antibodies raised to the α-subunit of inhibin.

Using *in situ* hybridisation with both rat and human DIG labelled riboprobes, mRNA for inhibin α-subunit was localised to the epithelial basal cells (Figure 6G) in 5 patients. In 1 out of 5 patients α mRNA was localised to basal and secretory epithelial cells (Figure 6H). The sense probe displayed no staining (Figure 6I and J). A summary of these results is presented in Table 5A.

EXAMPLE 13
BASAL CELL HYPERPLASIA

Tissue sections obtained from two patients with basal cell hyperplasia were used to detect inhibin α-subunit gene expression and protein localisation was determined. Identification of regions of basal cell hyperplasia was confirmed using a cytokeratin antibody as shown in Figure 7A. No immunoreactivity was localised in the control section (Figure 7B). αC and αN inhibin subunit protein immunoreactivity was also localised to these regions of the tissue sections and confirmed that inhibin proteins are localised to basal cells as shown in Figure 7C and E, respectively. No immunoreactivity was detected in the control sections (Figure 7D and F). The expression of inhibin α-subunit mRNA in basal cell hyperplasia was confirmed in one patient using *in situ* hybridisation (Figure 7G); no localisation was detected using the corresponding sense labelled riboprobe (Figure 7H).

EXAMPLE 14
PROSTATE CANCER

In 12 patients with poorly differentiated prostate cancer, the localisation of αC protein (9 patients), αN protein (6 of 12 patients) and α-inhibin mRNA (8 of 12 patients) was determined and compared in malignant and adjacent non-malignant regions of the tissues. As observed in tissue from patients with BPH, the αC protein was predominantly localised to the basal cells of non-malignant regions of tissue sections in 8 of 11 patient tissues (Figure 8A) and to basal and secretory cells in 3 of 11 patient tissues. In the adjacent poorly differentiated tumour tissue no positive immunoreactivity was observed (Figure 8B). Similarly, the pattern of staining of the αN to the basal cells of non-malignant regions of tissue sections in 8 of 11 patient tissues (Figure 8A) and to basal and secretory cells in 3 of 11 patient tissues. In the adjacent poorly differentiated tumour tissue no positive immunoreactivity was observed (Figure 8B). Similarly, the pattern of staining of the αN protein was predominantly localised to the basal and epithelial cells in the non-malignant region of tissue sections from men with advanced stage cancer of the prostate (Figure 8C): no immunoreactivity was observed in the adjacent tumour tissue (Figure 8D). The control for both the malignant and non-malignant regions displayed no positive staining (Figure 8E and F, respectively).

In situ hybridisation was performed using tissue from 8 patients with histological grate 4/5 prostate cancer and confirmed the pattern of protein inhibin localisation. Hence, α-subunit gene expression was detected in basal cells in 7 of 8 patients in non-malignant regions and in both basal and secretory cells in some patients (Figure 8G). Malignant tumour cells in adjacent regions of the same patient biopsies did not display any α-subunit gene expression (Figure 8H). No staining was observed with the inhibin α sense riboprobe (Figure 8I and J). The results for the non-malignant regions of the patient tissue are summarised in Table 5C.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Table 2 The effect of EDS, 3 and 14 days after administration, on prostate weight and the levels of ir-inhibin and ir-activin in the prostate, expressed as ng/g tissue and ng/organ. Values are means ± S.D., n=5

	3-Day control (DMSO)	**3-Day EDS**	**14-Day control** (DMSO)	**14-Day EDS**
Prostate weight	0·56 ± 0·15	0·38 ± 0·05	0·49 ± 0·07	0·07 ± 0·02*
ir-Inhibin				
ng/g tissue	19·1 ± 2·2	22·1 ± 3·4	15·7 ± 3·4	13·9 ± 4·4
ng/organ	11·1 ± 2·8	8·43 ± 1·9	7·6 ± 1·5	1·0 ± 0·4*
ir-Activin				
ng/g tissue	240 ± 156	277 ± 203	319 ± 224	264 ± 137
ng/organ	136 ± 86	101 ± 65	168 ± 145	17 ± 8*

*$P<0·01$ vs control (data analysed using ANOVA and Student-Newman-Keuls multiple range test).

Table 3 The levels of ir-inhibin and ir-activin measured by radioimmuoassay in normal adult rats testis and in normal and 3-day castrate rat prostate cytosols.
Values are means ± S.D., n=5 tissues

	Normal adult testis	Normal adult prostate	Prostate from 3-day castrate
Organ weight	1·596 ± 0·093	0·595 ± 0·070	0·294 ± 0·102*
ir-Inhibin			
ng/g tissue	9·10 ± 1·85	11·38 ± 3·03	15·91 ± 1·88
ng/organ	14·47 ± 2·76	6·89 ± 2·30	4·74 ± 1·95
ir-Activin			
ng/g tissue	162 ± 32	376 ± 59	574 ± 76
ng/organ	258 ± 50	223 ± 44	168 ± 61

*$P<0·01$ vs control (data analysed using ANOVA and Student-Newman-Keuls multiple range test).

TABLE 4. Sequence and PCR Conditions for Oligonucleotide Primers

Sequence	5' to 3' sequence	SEQ ID NO	Cycle conditions			Cycles
ActRII	CGG GAT CCA ACT GCT ATG ACA G	9	95°C	56°C	72°C	35
	GGA ATT CGC ACC AAR GAA CTG	10				
Follistatin	TTC CCT CTG TGA TGA GCT GTG	11	95°C	60°C	72°C	40
	AGC TGT AGT AGT CCT GGT CTT CAT	12				
α	CAT GCA GAC CTC TGA ACC AG	13	95°C	65°C		40
	GTG GCT GCG TAT GTG TTG GGA TG	14				
β_A	CTT GAA GAA GAC CCG ATG TCA C	15	95°C	53°C	71°C	40
	AAG AGG ATG GTG ACT TTG GTC	16				
β_B	GAA ATC ATC AGC TTC GCC GAG AC	17	95°C	57°C	71°C	50
	GAA CTG TTG CCT GCA ACA GAG GTT G	18				
β_C	ATG ACC TCC TCA TTG CTT CTG GC	19	95°C	53°C	71°C	50
	TTC ACA TTC CAG TTC CCT GTT GTC	20				

TABLE 5A and 5B. Summary of the positive expression and localization of inhibin α-subunit in tissue from men with benign prostatic hyperplasia (BPH) (A), basal cell hyperplasia (BCH) (B), and prostate cancer (PCA) (C)

	Patient No.	Immunoreactivity		
		α_N	α_c	*In Situ* ....
A.	BPH tissues			
	1	+bs		
	2	+bs		
	3	+bs		+b
	4	+bs	+b	
	5	+bs	+bs	
	6	+bs	+b	
	7		+b	
	8		+b	
	9		+b	
	10		+bs	
	11		+b	
	12			+b
	13			+bs
	14			+b
	15			+b
B.	BCH tissues			
	1	+b	+b	
	2	+b	+b	+b

+, Positive localization of protein or mRNA expression; b, staining in basal cells; s, staining in secretory epithelial cells; *, nonmalignant regions of tissue were not present in the sections used; only regions of tumor were present, so the localization in adjacent nonmalignant regions could not be described for these two patients. Note that not all patient tissues were used for the localization of α_C/α_N protein and mRNA expression; most tissues were used for either immunochemistry or *in situ* hybridization.

TABLE 5C. Summary of the positive expression and localization of inhibin α-subunit in tissue from men with benign prostatic hyperplasia (BPH) (A), basal cell hyperplasia (BCH) (B), and prostate cancer (PCA) (C)

	Patient No.	Immunoreactivity		
		α_N	α_c	*In Situ*
C.	1		+b	+b
	2	+bs	+b	+bs
	3		+b	+b
	4	*	*	+bs
	5	+bs	+b	+bs
	6	+bs	+b	
	7	+bs	+bs	+bs
	8	+bs	+bs	
	9	+bs	+b	*
	10		+bs	
	11		+b	+bs
	12		+b	+b

+, Positive localization of protein or mRNA expression; b, staining in basal cells; s, staining in secretory epithelial cells; *, nonmalignant regions of tissue were not present in the sections used; only regions of tumor were present, so the localization in adjacent nonmalignant regions could not be described for these two patients. Note that not all patient tissues were used for the localization of α_C/α_N protein and mRNA expression; most tissues were used for either immunochemistry or *in situ* hybridization.

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(v) COMPUTER READABLE FORM:
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(viii) ATTORNEY/AGENT INFORMATION:
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(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCCCAGCTC CTGGAAGGAG AT 22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCAGCCCAGC TGTGGTTCCA C 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGAATTCGCA CCAATGAACT G 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGGATCCAA CTGCTATGAC AGG 23

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAGGACAATG TGGCTTCGGG TGG 23

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGCCAGCACC GCGGTGAG 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGGATCCAA CTGCTATGAC AG 22

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGAATTCGCA CCAARGAACT G 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTCCCTCTGT GATGAGCTGT G 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGCTGTAGTA GTCCTGGTCT TCAT 24

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CATGCAGACC TCTGAACCAG 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGGCTGCGT ATGTGTTGGG ATG 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTGAAGAAG ACCCGATGTC AC 22

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AAGAAGATGG TGACTTTGGT C 21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAAATCATCA GCTTCGCCGA GAC 23

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAACTGTTGC CTGCAACAGA GGTTG 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATGACCTCCT CATTGCTTCT GGC 23

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TTCACATTCC AGTTCCCTGT TGTC 24

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CLAIMS:

1. A method of modulating cell growth in a mammal said method comprising administering to said mammal an effective amount of an agent for time and under conditions sufficient to modulate the expression of a genetic sequence encoding inhibin.

2. A method according to claim 1 wherein said cells are prostate cells.

3. A method according to claim 2 wherein said prostate cells are malignant.

4. A method according to claim 1 or 2 or 3 wherein said inhibin is α-inhibin.

5. A method according to claim 4 wherein said modulation of the expression of said genetic sequence is up-regulation.

6. A method according to claim 5 wherein said up-regulation inhibits cell growth.

7. A method of modulating cell growth in a mammal said method comprising administering to said mammal an effective amount of inhibin.

8. A method according to claim 7 wherein said cells are prostate cells.

9. A method according to claim 8 wherein said prostate cells are malignant.

10. A method according to claim 7 or 8 or 9 wherein said inhibin is α-inhibin.

11. A method according to claim 10 wherein said modulation of cell growth is inhibition of cell growth.

12. A method of modulating cell growth in a mammal said method comprising administering to said mammal an effective amount of an inhibin antagonist.

13. A method according to claim 12 wherein said cells are prostate cells.

14. A method of treating a mammal said method comprising administering to said mammal an effective amount of an agent for a time and under conditions sufficient to modulate the expression of a genetic sequence encoding inhibin.

15. A method according to claim 14 wherein said cells are prostate cells.

16. A method according to claim 15 wherein said prostate cells are malignant.

17. A method according to claim 14 or 15 or 16 wherein said inhibin is α-inhibin.

18. A method according to claim 17 wherein said modulation of the expression of said genetic sequence is up-regulation.

19. A method according to claim 18 wherein said up-regulation inhibits cell growth.

20. A method of treating a mammal said method comprising administering to said mammal an effective amount of inhibin.

21. A method according to claim 20 wherein said cells are prostate cells.

22. A method according to claim 21 wherein said prostate cells are malignant.

23. A method according to claim 20 or 21 or 22 wherein said inhibin is α-inhibin.

24. A method according to claim 23 wherein said modulation of cell growth is inhibition of cell growth.

25. A method of treating a mammal said method comprising administering to said mammal an effective amount of an inhibin antagonist.

26. A method according to claim 25 wherein said cells are prostate cells.

27. The use of an agent capable of modulating the expression of a genetic sequence encoding inhibin in the manufacture of a medicament for the modulation of cell growth in a mammal.

28. Use of an agent according to claim 27 wherein said cells are prostate cells.

29. Use of an agent according to claim 28 wherein said prostate cells are malignant.

30. Use of an agent according to claim 27 or 28 or 29 wherein said inhibin is α-inhibin.

31. Use of an agent according to claim 30 wherein said modulation of the expression of said genetic sequence is up-regulation.

32. Use of an agent according to claim 31 wherein said up-regulation inhibits cell growth.

33. Use of inhibin in the manufacture of a medicament for the modulation of cell growth in a mammal.

34. Use of inhibin according to claim 33 wherein said cells are prostate cells.

35. Use of inhibin according to claim 34 wherein said prostate cells are malignant.

36. Use of inhibin according to claim 33 or 34 or 35 wherein said inhibin is α-inhibin.

37. Use of inhibin according to claim 36 wherein said modulation of cell growth is inhibition of cell growth.

38. Use of an inhibin antagonist in the manufactue of a medicament for the modulation of cell growth in a mammal.

39. Use of an inhibin antagonist according to claim 38 wherein said cells are prostate cells.

40. An agent for use in modulating the expression of a genetic sequence encoding inhibin wherein modulating expression of said genetic sequence modulates cell growth.

41. An agent according to claim 40 wherein said cells are prostate cells.

42. An agent according to claim 41 wherein said prostate cells are malignant.

43. An agent according to claim 40 or 41 or 42 wherein said inhibin is α-inhibin.

44. An agent according to claim 43 wherein said modulation of the expression of said genetic sequence is up-regulation.

45. An agent according to claim 44 wherein said up-regulation inhibits cell growth.

46. An agent for use in the modulation of cell growth in a mammal comprising inhibin.

47. An agent according to claim 46 wherein said cells are prostate cells.

48. An agent according to claim 47 wherein said prostate cells are malignant.

49. An agent according to claim 46 or 47 or 48 wherein said inhibin is α-inhibin.

50. An agent according to claim 49 wherein said up-regulation inhibits cell growth.

51. An agent for use in the modulation of cell growth in a mammal comprising an inhibin antagonist.

52. An agent according to claim 51 wherein said cells are prostate cells.

53. A pharmaceutical composition comprising an agent capable of modulating expression of a genetic sequence encoding inhibin thereby modulating cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

54. A claim according to claim 53 wherein said inhibin is α-inhibin.

55. A pharmaceutical composition comprising inhibin capable of modulating cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

56. A pharmaceutical composition according to claim 55 wherein said inhibin is α-inhibin.

57. A pharmaceutical composition comprising an inhibin antagonist capable of modulating cell growth and one or more pharmaceutically acceptable carriers and/or diluents.

58. A method of screening for a mammal having prostate cancer or predisposition to prostate cancer, said method comprising screening for the down-regulation of inhibin protein levels in said mammal wherein the down-regulation of said inhibin protein levels is indicative of said mammal being predisposed to prostate cancer or having already developed prostate cancer.

59. A method of screening for a mammal having prostate cancer or a predisposition to prostate cancer, said method comprising screening for the down-regulation of inhibin gene expression in said mammal wherein the down-regulation of said inhibin gene expression is indicative of said mammal being predisposed to prostate cancer or having already developed prostate cancer.

60. The method according to claim 58 or 59 wherein said inhibin is α-inhibin.

61. The method according to claim 60 wherein said α-inhibin is αN or isoform thereof.

62. The method according to claim 60 wherein said α-inhibin is αC isoform thereof.

63. The method according to claim 58 or 59 or 60 or 61 or 62 wherein said down-regulation is absence.

64. A method of screening for a mammal having prostate cancer said method comprising screening for the down-regulation of α-subunit gene expression in said individual, wherein the down-regulation of α-subunit gene expression is indicative of prostate cancer.

65. The method according to claim 64 wherein said down-regulation is absence.

66. A method of screening for a mammal having a predisposition to prostate cancer, said method comprising screening for α-subunit gene expression in said individual wherein α-subunit gene expression reveals disruption of the basement membrane, said disruption indicating a predisposition to prostate cancer.

67. A method of screening for a mammal having prostate cancer or a predisposition to prostate cancer, said method comprising screening for the modulation of the activin protein in said mammal wherein modulation of the activin protein is indicative of said mammal being predisposed to prostate cancer or having already developed prostate cancer.

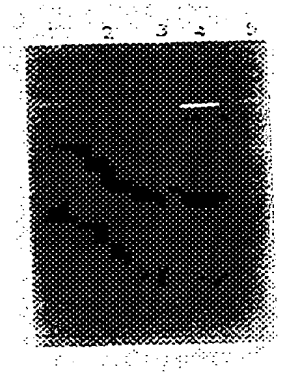


FIGURE 1

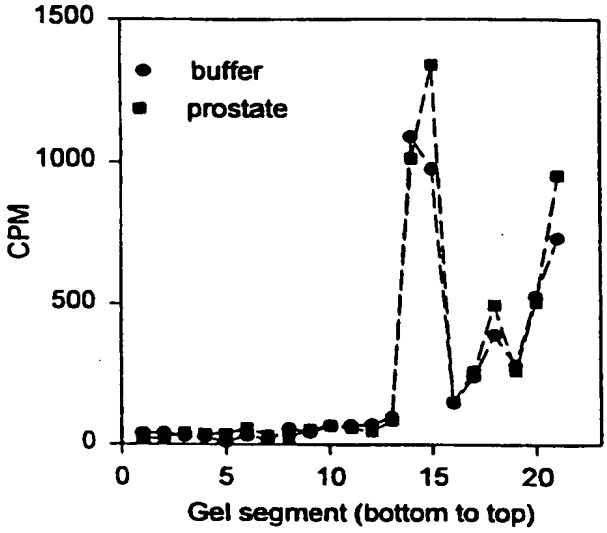


FIGURE 2b

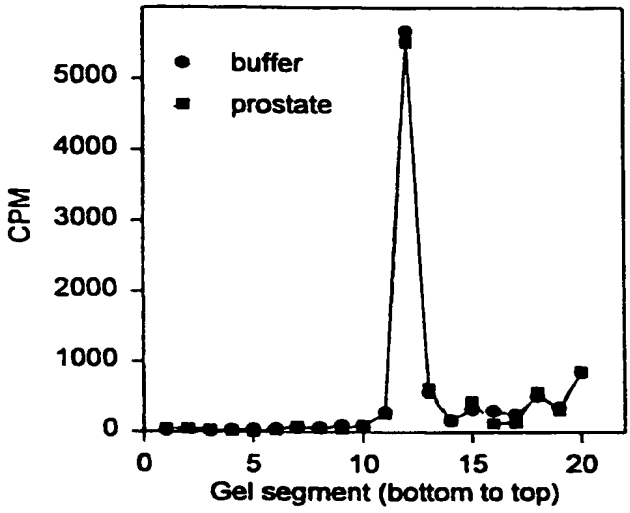


FIGURE 2a

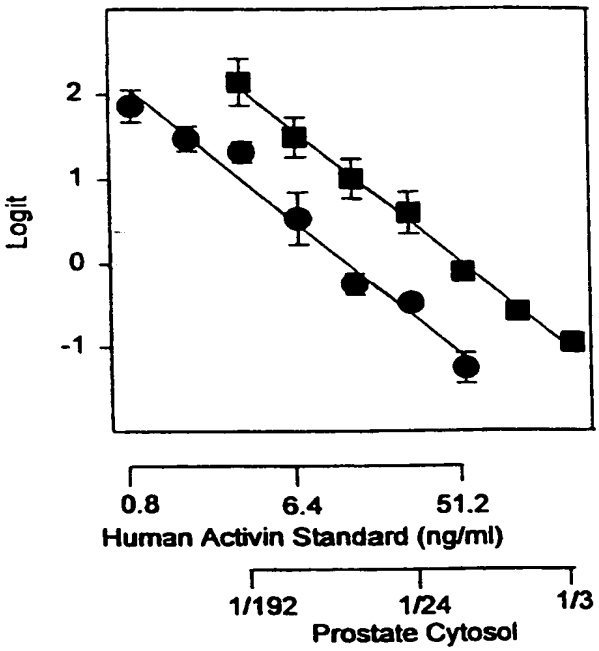

2
1
0
-1
Logit
0.8
6.4
51.2
Human Activin Standard (ng/ml)
1/192
1/24
1/3
Prostate Cytosol

FIGURE 3

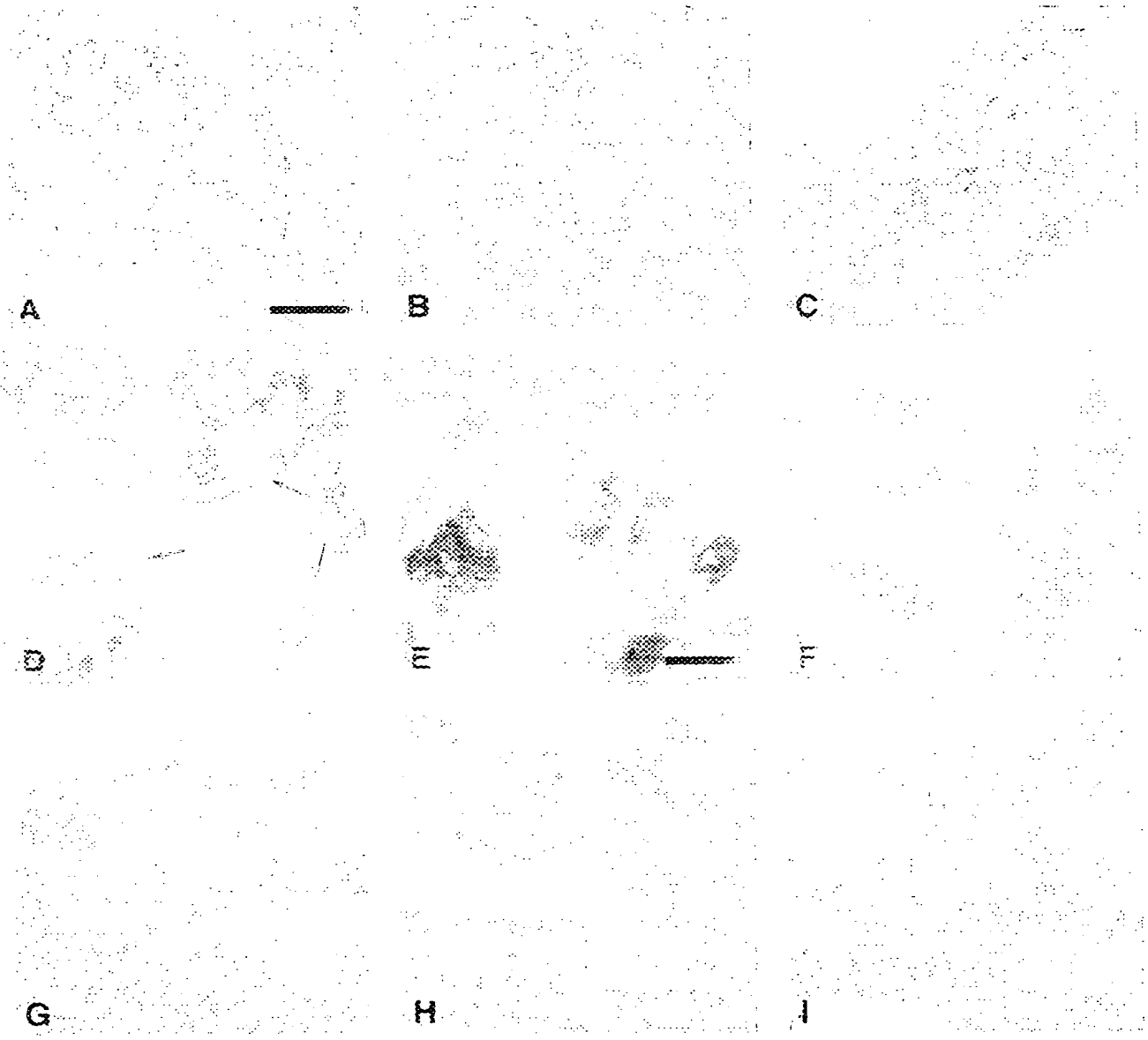
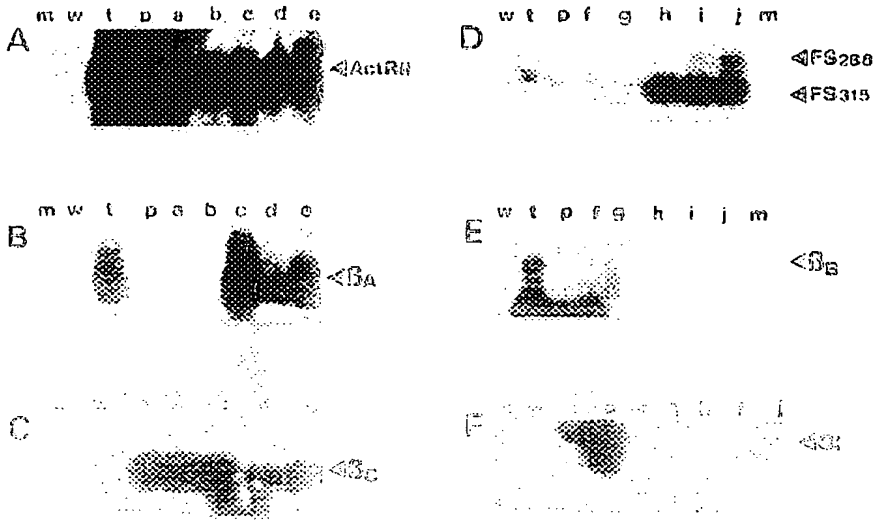


FIGURE 4


A
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ActRII
B
m w t p a b c d e
βA
C
βC
D
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w t p f g h i j m
βB
F
βA

FIGURE 5

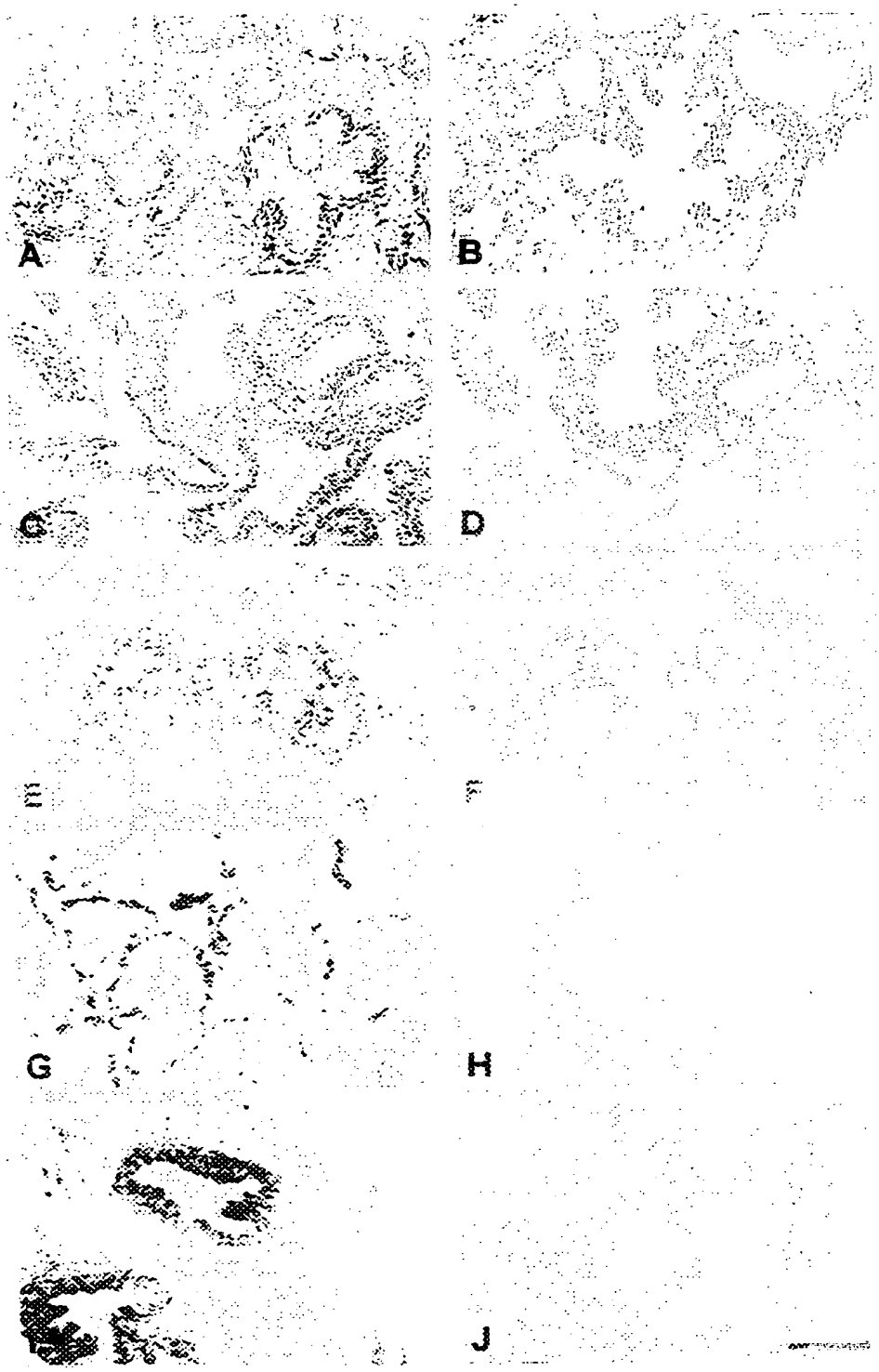

FIGURE 6

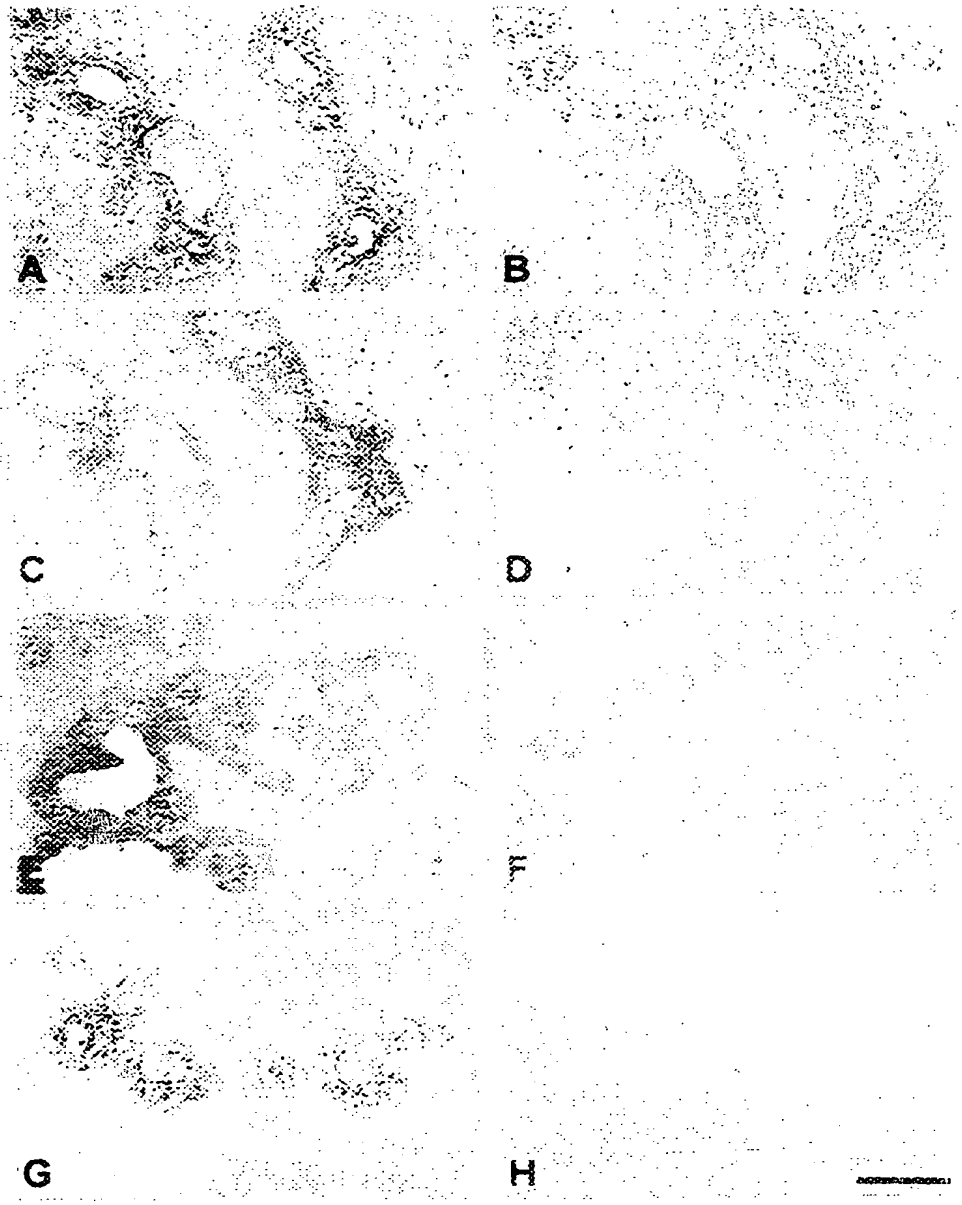

FIGURE 7

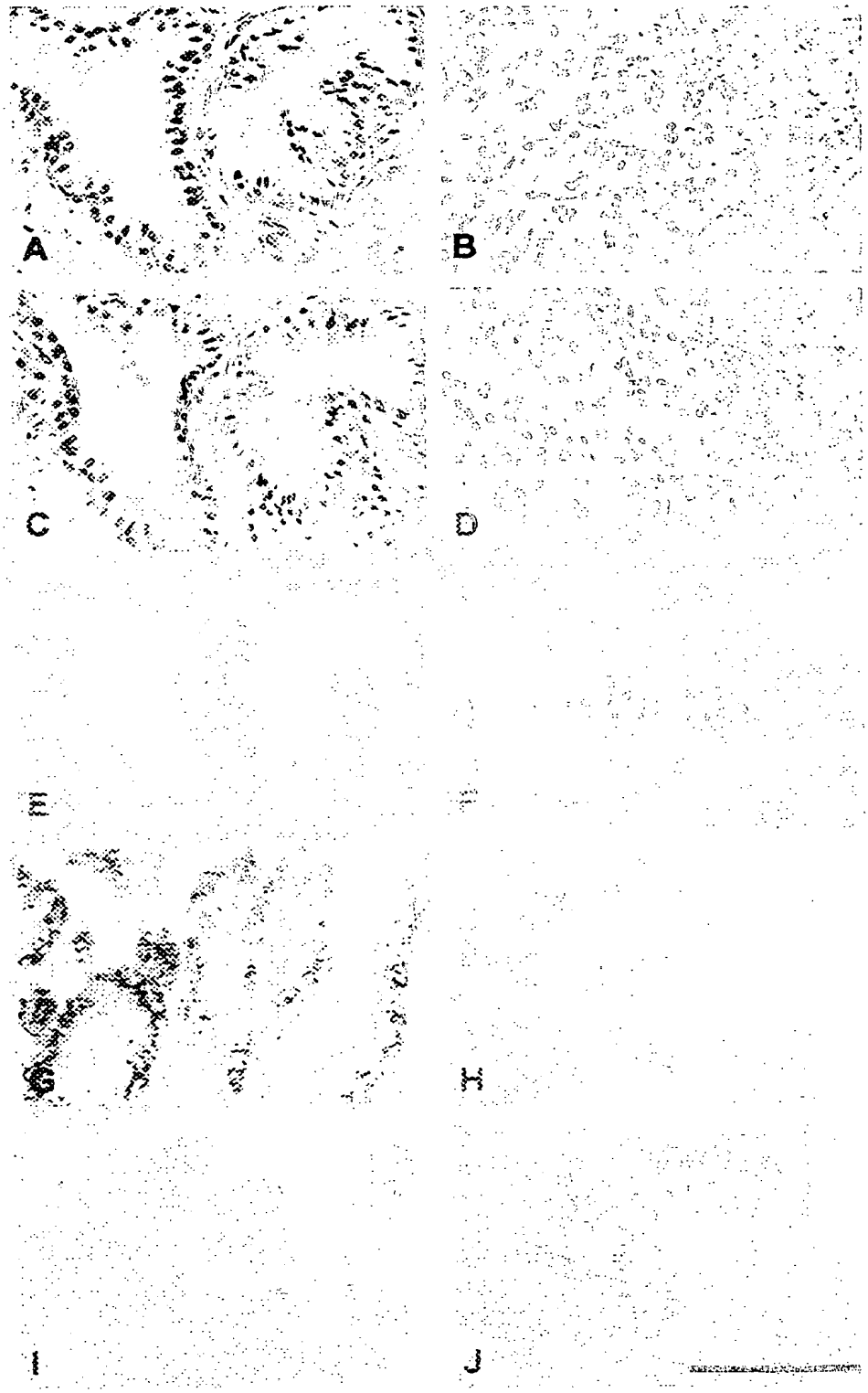

FIGURE 8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00292

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 38/22, G01N 33/74, C12Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC A61K, G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: inhibin; activin and prostat:
MEDLINE: inhibin and prostate and growth; (inhibin* or activin) and prostatic neoplasms
CAPLUS: inhibin/RN/THU and prostat?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93/25224 A (VETROGEN CORPORATION) 23 December 1993 entire document	1-67
X	WO 89/11862 A (BIOTECHNOLOGY AUSTRALIA PTY. *et al.*) 14 December 1989 entire document	1-57
X	WO 86/06076 A (BIOTECHNOLOGY AUSTRALIA PTY. LTD. *et al.*) 23 October 1986 entire document	1-57

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
20 May 1998

Date of mailing of the international search report
27 MAY 1998

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
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AUSTRALIA
Facsimile No.: (02) 6285 3929

Authorized officer
T. SUMMERS
Telephone No.: (02) 6283 2291

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00292

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 86/00078 A (MONASH UNIVERSITY *et al.*) 3 January 1986 entire document	1-57
X	WO 91/10445 A (GENETECH, INC.) 25 July 1991 entire document	1-57
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